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CONTENTS

NUMBER 1, AUGUST, 1945

nylenes, Dichromylenes, Dixanthylenes, and their Sulfur Analogues. ALEXANDER SCHÖNBERG AND (MISS) WAFFIA ASKER.....	1
emical Genetics. G. W. BEADLE	15
Basicity Characteristics of Scandium, Yttrium, and the Rare Earth Elements. THERALD MOELLER AND HOWARD E. KREMERS.....	97

NUMBER 2, OCTOBER, 1945

ical Characteristics and Physiological Rôles of Glutamine. REGINALD A. ARCHIBALD.....	161
nyl Bridge Compounds and Related Substances. C. F. H. ALLEN ..	209
Chemistry of Cinnolines. NELSON J. LEONARD.....	269
id Chemistry of Clays. ERNST A. HAUSER.....	287

NUMBER 3, DECEMBER, 1945

Alkylolation of Alkanes. GUSTAV EGLOFF AND GEORGE HULLA	323
Chemistry of the Oxazoles. RICHARD H. WILEY.....	401
Chemistry of Oxindole. WARD C. SUMPTER.....	443
ietic Estrogens and the Relation Between their Structure and their Activity. ULRICH V. SOLMSEN.....	481

DIPYRYLENES, DICHROMYLENES, DIXANTHYLENES, AND THEIR SULFUR ANALOGUES

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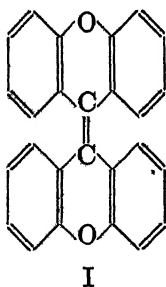
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CONTENTS

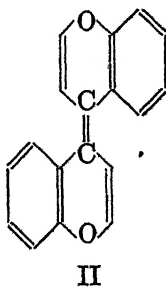
I. Introduction	1
II. Methods of preparation	2
A. By the thermal decomposition of thioketones	2
B. By the action of copper on aromatic thioketones	4
C. By the action of diazomethane with thioketones, followed by the action of lithium phenyl	5
D. By the reduction of ketones	6
E. By the action of copper bronze on keto chlorides	7
F. By the action of sodium on xanthone diphenyl mercaptole	8
III. Chemical properties	8
A. Action with oxidizing and reducing agents	8
B. Action with halogens and with phosphorus pentachloride	9
C. Action with acids	10
D. Action with alkalis	10
E. Action with sodium and potassium	10
F. Action with sulfur	10
G. Action with thionyl chloride	11
IV. The color of dipyrylenes, dichromylenes, and dixanthylenes	11
V. References	13

I. INTRODUCTION

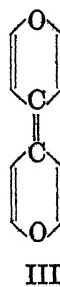
Whereas dixanthylene (I) (14) and dithioxanthylene (IV) (19) were synthesized many years ago, dichromylenes (II) (32) and dipyrylenes (III) (2) have only recently been discovered. The same is true for dithiochromylenes (V) (33) and dithioppyrylenes (VI) (3).



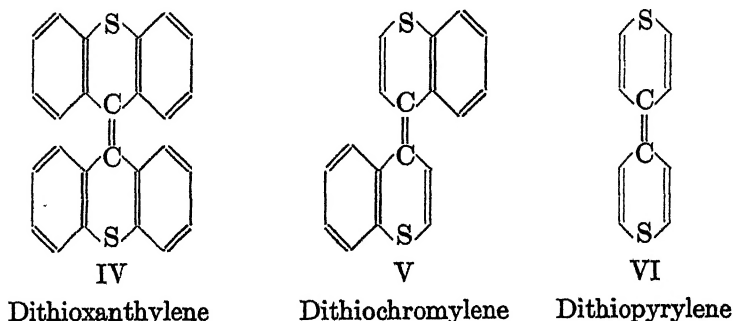
I
Dixanthylene



II
Dichromylene



III
Dipyrylene

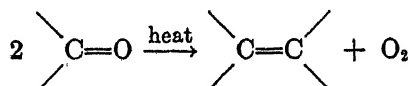


Cis-trans isomers of dichromylenes and dipyrylenes (and their sulfur analogues) have not yet been found with certainty. According to Taylor (38) 2,2'-diphenyl-dichromylene (diflavylene) (XXVII) (page 5) has the *trans*-form. Formulas II and V represent the *trans*-forms of dichromylene and dithiochromylene. The present review summarizes the methods of preparation and chemical properties of these compounds. A discussion of the theories concerning their color is also given.

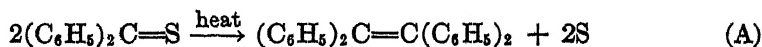
II. METHODS OF PREPARATION

A. By the thermal decomposition of thioketones

Although it has not been observed that ketones change at ordinary temperature or at temperatures below 200°C. according to the following scheme:



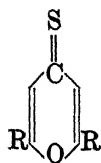
the analogous process has been observed in the case of thioketones. Thiobenzophenone is transformed to tetraphenylethylene when heated at 160–170°C., according to the following scheme (37):



Many derivatives of thiobenzophenone are stable towards heat; for example, xanthione (XX) (page 4) is not changed when heated to 220°C. for 4 hr. (4); also in the 4-thiochrome series (see compound XXIVa) reactions analogous to that in equation A have not yet been observed. But Arndt (2) found that diethyl 4-thiochelonate (IX) is transformed into ethyl 4,4'-dipyrylenetetracarboxylate (XII) at high temperatures. The reaction takes place very slowly at room temperature and is accelerated by the influence of light.

It has also been found that 2,6-diphenyl-4-thiopyrone (X) reacts similarly to compound IX, giving 2,2',6,6'-tetraphenyl-4,4'-dipyrylene (XIII) (4). 2,6-Dimethyl-4-thiopyrone (VIII) and 4-thiopyrone (VII) do not react according to equation A (4), but 2,6-diphenyl-1,4-dithiopyrone (XI) when heated at 145°C. gives tetraphenyldithiopyrylene (XIV) (3).

DIPYRYLENES, DICHROMYLENES, AND DIXANTHYLENES

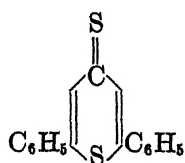


VII, R = H

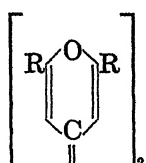
VIII, R = CH₃

IX, R = COOC₂H₅

X, R = C₆H₅

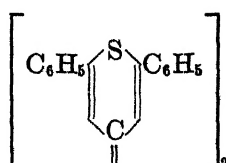


XI



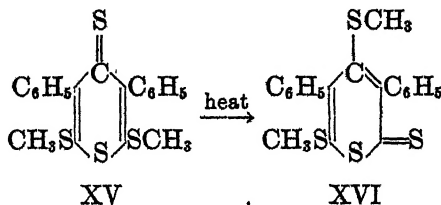
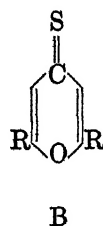
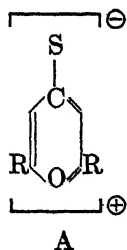
XII, R = COOC₂H₅

XIII, R = C₆H₅



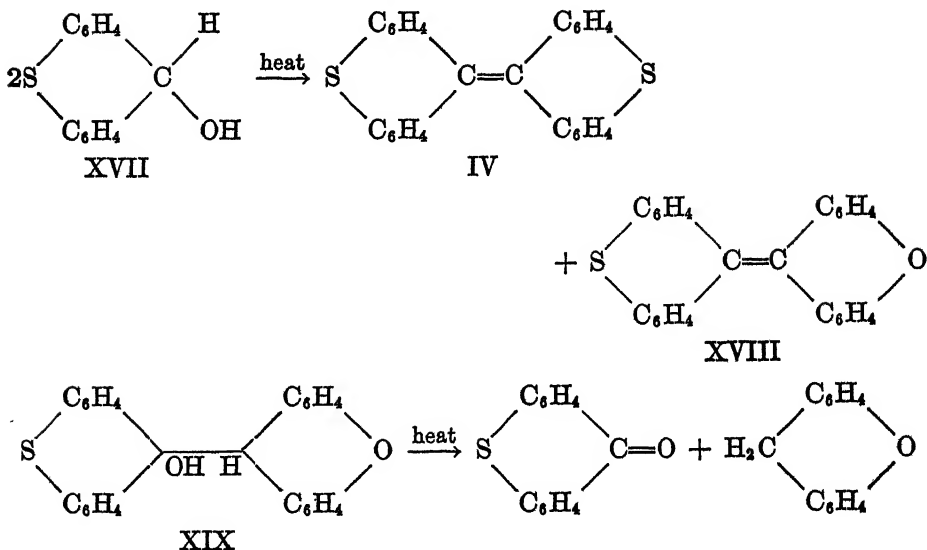
XIV

Arndt (4) investigated the relation between the constitution of the thiopyrones and their stability towards heat and concluded that 4-thiopyrones change into dipyrylenes, provided they carry an acidifying substituent such as —COOC₂H₅ or —C₆H₅ in the α -position, and that such a change is easier when the group is more acidic. This is probably due to the basic properties of the pyrone oxygen ring, which can be weakened or neutralized by the introduction of acidic groups in the 2- and 6-positions. The compound tends to acquire more or less the constitution shown in formula B and to behave therefore as a real thioketone which undergoes the "dipyrylene reaction." The thermostable 2,6-dimethyl-4-thiopyrone (XIII) has more the character of a betain (formula A).



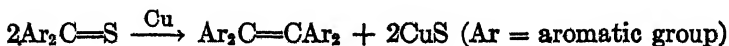
When the thioketone (XV) was heated, no loss of sulfur was observed, but an isomeric change took place. The constitution of the new compound is believed to be as shown in formula XVI (27).

Dithioxanthylene (IV) has been obtained by the action of heat on thioxanthohydrol (XVII) (30). An attempt was made to obtain substance XVIII by the elimination of water from XIX by heat, but a mixture of thioxanthone and xanthene was formed instead (31).

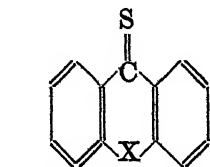


B. By the action of copper on aromatic thioketones

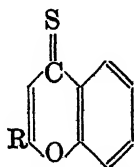
Thiobenzophenone and some of its derivatives are transformed by the action of heavy metals (for example, copper) to the corresponding ethylene derivatives. According to Gattermann (11, 12), who first carried out the reaction, a mixture of certain thioketones and copper powder (obtained by the reduction of pure copper oxide with hydrogen) when heated at 200–220°C. for 15–30 min. in a stream of carbon dioxide yields the corresponding ethylene derivatives and cupric sulfide.



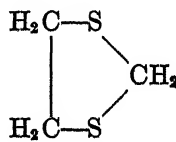
Schönberg (36) and coworkers modified the process and obtained a purer product by refluxing the thioketone with copper bronze in toluene or xylene solution. The different behavior of the thioketones towards copper bronze is as follows: thiobenzophenone reacts readily, *p,p'*-dimethoxythiobenzophenone, xanthione (XX), and thioxanthione (XXI) less readily, whereas *N*-phenylthioacridone (XXII) and Michler's thioketone are very resistant.



XX, X = O
XXI, X = S
XXII, X = NC₆H₅
XXIII, X = NCH₃



XXIV, R = C₆H₅
XXIVa, R = H

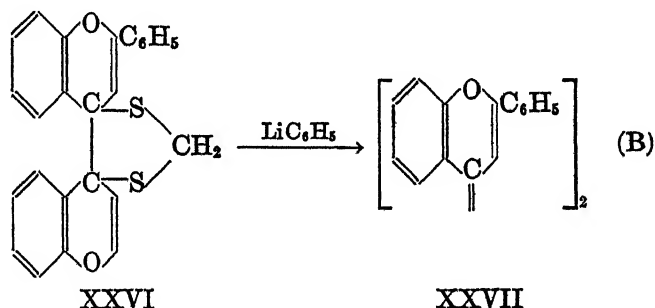
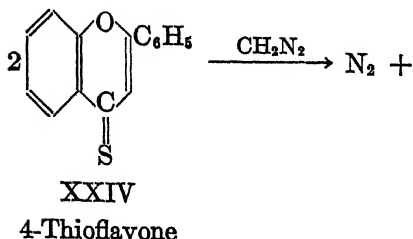


XXV

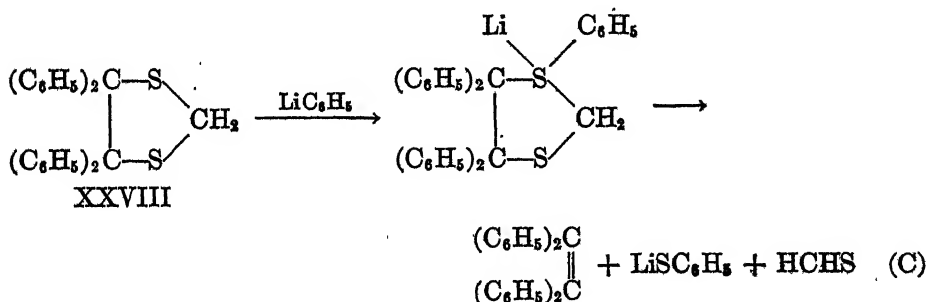
Diflavylene (2,2'-diphenyldichromylene) (XXVII) can also be obtained from 4-thioflavone (XXIV), but the yield is not good (29).

C. By the action of diazomethane on thioketones, followed by the action of lithium phenyl

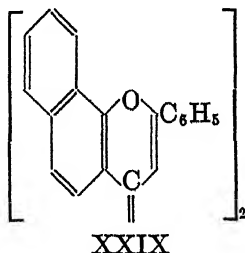
Derivatives of trimethylene 1,3-disulfide (XXV)—for example, compound XXVI—are obtained by the action of diazomethane on thioketones. Scheme B represents the action of diazomethane on 4-thioflavone (XXIV), according to Schönberg (32).



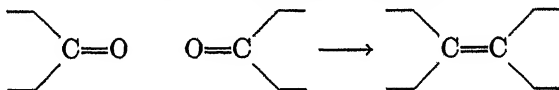
Such products are of special interest, for they are decomposed by lithium phenyl into the corresponding ethylene derivatives with the formation of lithium thiophenolate ($\text{C}_6\text{H}_5\text{SLi}$) and thioformaldehyde. The action of lithium phenyl on XXVI, leading to the formation of diflavylene (XXVII), is similar to the action of lithium phenyl on 4,4',5,5'-tetraphenyltrimethylene 1,3-disulfide (XXVIII), which leads to the formation of tetraphenylethylene according to scheme C (29):



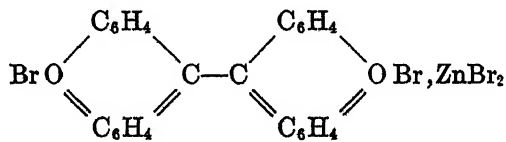
By this method not only dichromylenes (32) have been prepared, but also dixanthylene (I), dithioxanthylene (IV), and the naphthalene product (XXIX) (29):



D. By the reduction of ketones

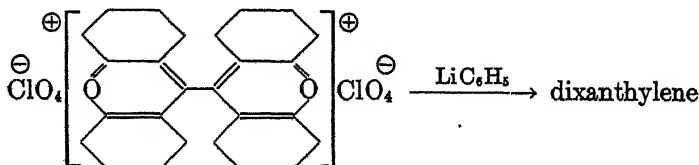


Xanthone and some of its derivatives when boiled with acetic acid and zinc dust in the presence of some drops of hydrochloric acid are converted into dixanthylene (I) or the corresponding dixanthylene derivatives (14). By the reduction of xanthone with zinc and hydrobromic acid, dixanthonium bromide zinc bromide is obtained (39).

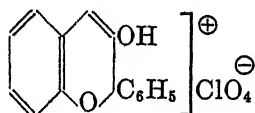


The reduction of thioxanthone with zinc and hydrochloric acid gives dithioxanthylene (IV) (19). Baeyer and Piccard (5, 6) reduced 2,6-dimethylpyrone with zinc dust and hydrochloric acid, but did not isolate the corresponding tetramethyldipyrlylene.

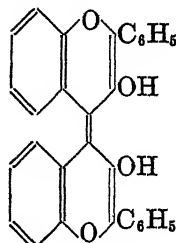
The reduction of xanthone with a mixture of zinc wool and sodium perchlorate in acetic acid and acetic anhydride yields red crystals of 9,9-dihydroxybixanthyl perchlorate, which reacts with lithium phenyl or with phenylmagnesium bromide giving dixanthylene (33):



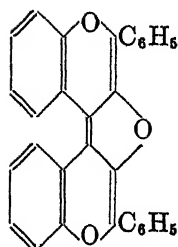
Dilthey (9) heated 3-hydroxyflavenium perchlorate (XXX) with sodium acetate in ethyl alcohol; the intermediate compound 3,3-dihydroxy-4,4-diflavylene (XXXI) was obtained and converted into 4,4-diflavylene 3,3-oxide (XXXII) by the action of hot acetic acid, but not by sulfuric acid. The mother liquor of XXXII contained (4,4-diflavylene 3,3-oxide)-4,4-glycol (XXXIII).



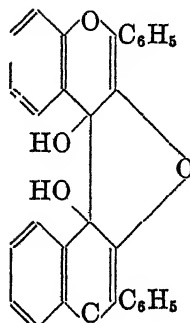
XXX



XXXI



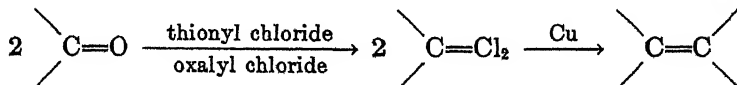
XXXII



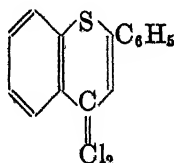
XXXIII

E. By the action of copper bronze on keto chlorides

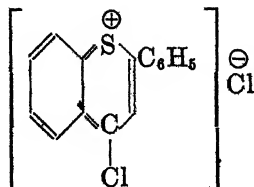
Schönberg and coworkers (25, 33) treated the ketones (for example, 1-thioflavone in the case of dithioflavylene (XXXVII)) with thionyl chloride or oxalyl chloride in the absence of moisture; the keto chlorides were obtained in good yield, and these in turn when refluxed with copper bronze in benzene solution gave the corresponding ethylene derivatives according to the following scheme:



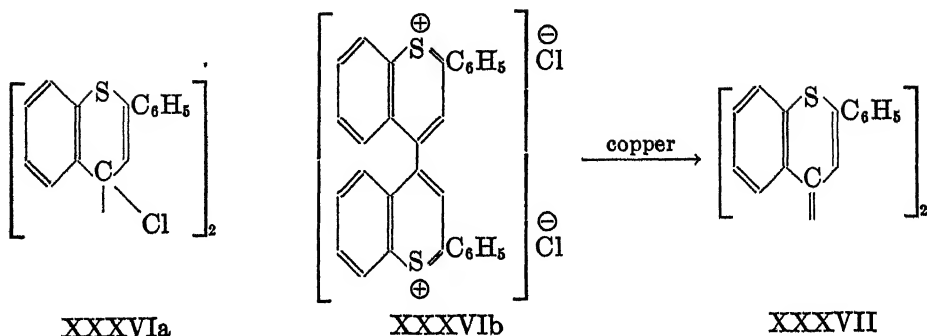
It must be pointed (25) out that it may be more correct to represent the products obtained by the action of thionyl chloride on chromones, pyrones, and xanthenes as salts. For example, the product obtained by the action of thionyl chloride on 1-thioflavone can be represented by formula XXXV instead of XXXIV. A compound (XXXVIa or b) was obtained by the action of copper bronze on XXXIV or XXXV, which must be regarded



XXXIV



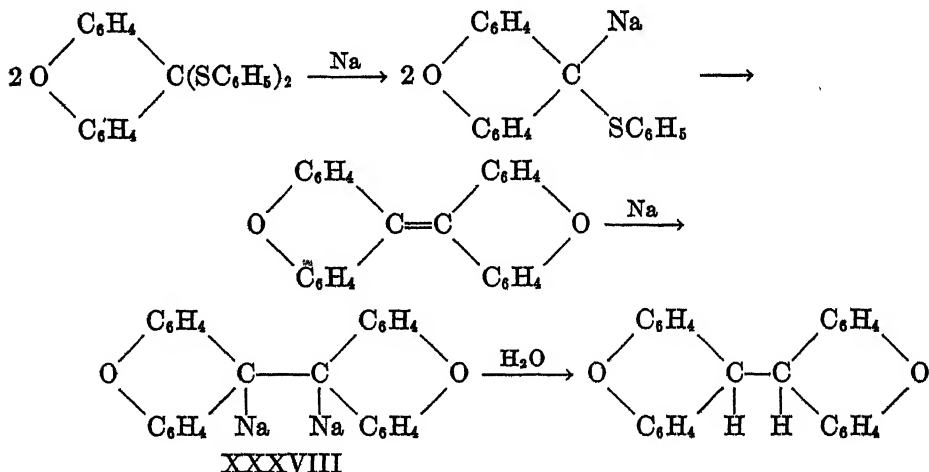
XXXV



as an intermediate product in the preparation of dithioflavylene (XXXVII), since it yields dithioflavylene on further treatment with copper bronze.

F. By the action of sodium on xanthone diphenyl mercaptole

Schönberg (34) obtained dixanthylene, together with dixanthyl, by the action of sodium on xanthone diphenyl mercaptole. The reaction proceeds according to the following scheme:



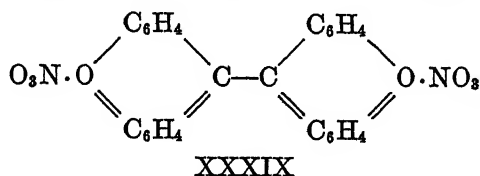
A similar reaction is brought about by the action of sodium on benzophenone diphenyl mercaptole.

III. CHEMICAL PROPERTIES

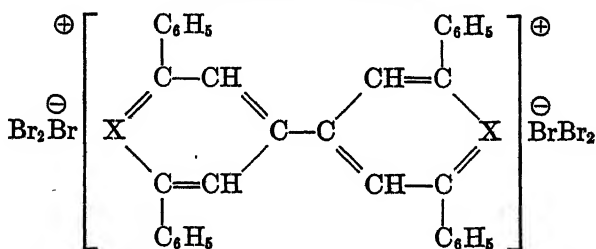
A. Action with oxidizing and reducing agents

It was found by Arndt (1) that dithioxanthylene (IV) is oxidized by hydrogen peroxide in acetic acid to dithioxanthylene disulfone. Dithioflavylene (XXXVII) and dithioxanthylene (IV) were not changed when air was passed through their solutions in thiophene-free benzene for 10 hr. at room temperature, but dixanthylene (I) was broken down at 300°C. into xanthone by the action of air in the presence of selenium (27). Dixanthylene (I) was converted by Werner (39)

into dioxanthonium nitrate (XXXIX), by suspending it in benzene and saturating the suspension with nitrous fumes at 60–70°C. The nitrate is a brown crystal-



Dioxanthonium nitrate



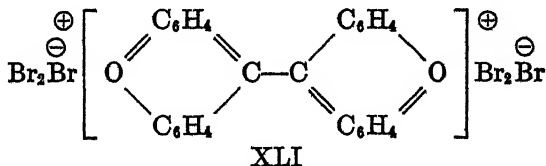
XL, X = O or S

line substance with bluish lustre, and is converted by hydrobromic acid into the corresponding bromide.

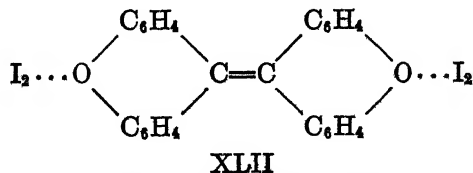
Very little is known about the behavior of the substances mentioned in the title of this paper towards reducing agents.

B. Action with halogens and with phosphorus pentachloride

When a solution of tetraphenyldipyrrole (XIII) or of its sulfur analogue (XIV) in chloroform is mixed with a solution of bromine in the same solvent, XL is formed (1, 4). Dixanthylene hexabromide (XLI) is formed by mixing the solution of dixanthylene in carbon disulfide with a solution of bromine in the same solvent (4, 15). It regenerates dixanthylene (I) when treated with an aqueous solution of sulfurous acid (15). Dixanthylene tetraiodide (XLII) is



Dixanthylene hexabromide



Dixanthylene tetraiodide

obtained when a solution of dixanthylene in carbon disulfide is mixed with a solution of iodine in the same solvent. The iodine is removed by the action of an alcoholic solution of sodium thiosulfate. When the solution of bromine in carbon tetrachloride is mixed with a solution of dithioxanthylene (IV) in *o*-dichlorobenzene, dithioxanthonium perbromide ($C_{26}H_{16}S_2Br_6$) is formed and dithioxanthylene is regenerated on treatment with zinc dust and acetic acid. Dithioxanthylene disulfone does not react with bromine solution or vapor even when heated (1).

Magidson and Damaskina (18) heated dixanthylene with phosphorus pentachloride and obtained 9,9-dichloroxanthene.

C. Action with acids

The substances mentioned in the title of this paper seem in general to be very stable towards acids as far as the ring system characteristic for them is concerned. Ethyl 4,4'-dipyrylenetetracarboxylate (XII) dissolves in cold concentrated sulfuric acid and on the addition of water, a great part of the ester is recovered unchanged (2). Tetraphenyldipyrlylene (XIII) is stable towards aqueous acids (4); dithioflavylene (XXXVII) is stable towards an aqueous solution of hydrochloric acid (27); dixanthylene (I) and dithioxanthylene (IV) are stable towards acids, since they are prepared in a strong acid medium (14, 19).

D. Action with alkalis

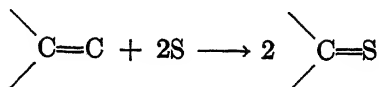
The stability of the ring system of the substances in question is illustrated by the following facts: Ethyl 4,4'-dipyrylenetetracarboxylate (XII) when boiled with an alcoholic solution of sodium hydroxide gives the sodium salt, but no break in the molecule takes place (2). Tetraphenyldipyrlylene (XIII) is completely stable towards aqueous alkalis (4); tetraphenyldithiopyrlylene (XIV) is also stable towards an alcoholic solution of sodium hydroxide (25).

E. Action with sodium and potassium

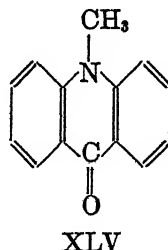
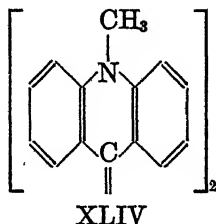
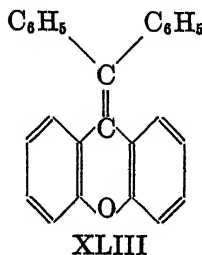
By the action of sodium on dixanthylene (I) the sodium salt (XXXVIII) is obtained, which gives 9,9'-dixanthyl-9,9'-dicarboxylic acid by the action of carbon dioxide followed by that of acids (22). Conant and Garvey (7) obtained the potassium salt by the action of a sodium-potassium alloy on dixanthylene.

F. Action with sulfur

It has been shown by Schönberg (23) that the ethylene linkage in dixanthylene (I) is split by the action of elementary sulfur with the formation of xanthione (XX). This reaction takes place very quickly at 280°C., 2 min. being sufficient (25). This was the first example of the splitting of a double bond by sulfur; later (25) it was shown that diflavylene (XXVII), dithioflavylene (XXXVII), dithioxanthylene (IV), and *o,o'*-oxido-tetraphenylethylene (XLIII) (28) are also broken down readily by sulfur to the corresponding thioketones at 280°C., according to the scheme:



Tetraphenyldithiopyrylene (XIV) does not undergo this reaction (25). This is explained by the fact that 1,4-dithio-2,6-diphenylpyrone (XI), which should be the product of this reaction, loses sulfur easily at 145°C. with the formation of XIV (3).

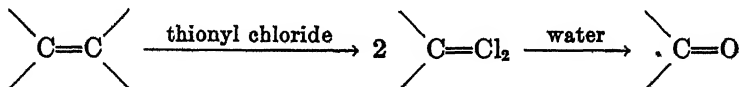


N,N-Dimethyldiacridene *N*-Methylacridone

A similar reaction (13) has been carried out with *N,N'*-dimethyldiacridene (XIV), which is an analogue of dixanthylene (I).

G. Action with thionyl chloride

The central ethylene linkage in diflavylene (XXVII), dithioflavylene (XXXVII), dixanthylene (I), dithioxanthylene (IV) (26), and *o,o'*-oxido-tetraphenylethylene (XLI) (28) can easily be split, by refluxing these substances with thionyl chloride, to the corresponding keto chlorides according to the scheme:

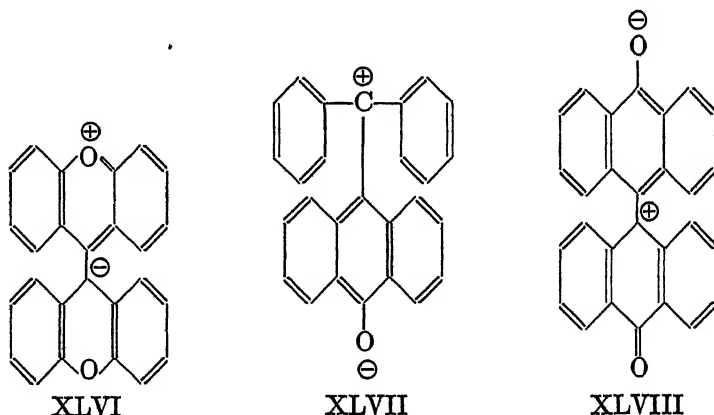


If, for example, dixanthylene is boiled with thionyl chloride, it gives an oil which on treatment with water at 30°C. yields xanthone in practically quantitative yield; when aniline is used instead of water, xanthone anil is obtained (26). *o,o'*-Oxido-tetraphenylethylene (XLI) gives a mixture of xanthone and benzophenone (28). *N,N'*-Dimethyldiacridene (XIV) gives *N*-methylacridone (XLV). Tetraphenylethylene when treated with thionyl chloride and water, as described in the case of dixanthylene, was practically unchanged (26).

IV. THE COLOR OF DIPYRYLENES, DICHROMYLENES, AND DIXANTHYLENES

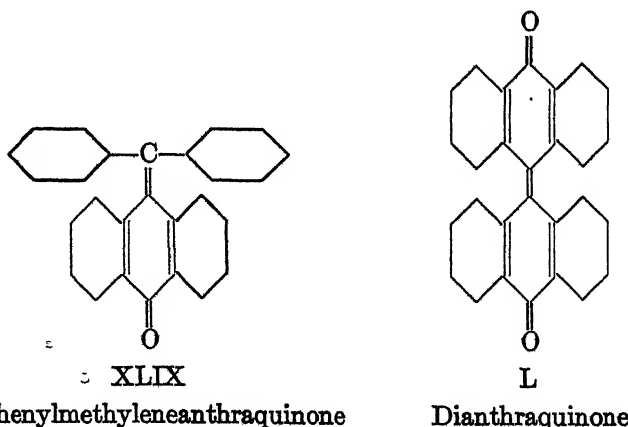
All dipyrylenes, dichromylenes, and their sulfur analogues are brilliantly colored substances. Dithioxanthylene (IV) is almost colorless and its melt is colorless (1). Dixanthylene (I) in the crystalline state is colorless at the temperature of liquid air; it is slightly colored at room temperature and gives a very bright blue melt; its solutions in indifferent solvents are bright blue green (23, 24).

To explain this phenomenon, it is suggested that dioxanthylene is a true ethylene in the crystalline state, especially at low temperatures, as indicated by formula I, but that in the melt and in solution the molecules acquire the structure of betains (XLVI).

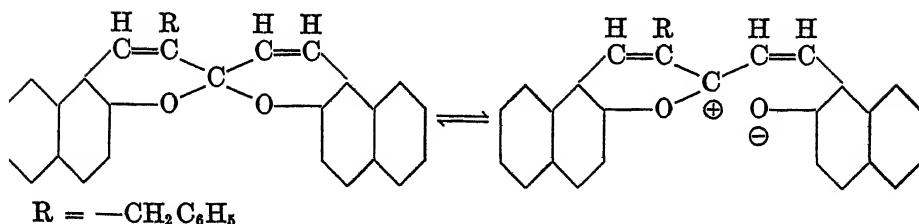


It is perhaps more correct to state that the molecule of dioxanthylene is to be regarded as a resonance hybrid with contribution *inter alia* from the true ethylene structure and the betain structure, and that there is a large contribution from the betain structure when the substance is in the melted state or in solution, and a small contribution under these conditions from the true ethylene form. The reverse is the case with regard to the molecules of the crystallized dioxanthylene (28).

Similar changes of color with temperature (thermochromism) have been observed *inter alia* with diphenylmethylenanthraquinone (XLIX) (21) and dianthraquinone (L) (20) and may be explained similarly (compare the betain formulas XLVII and XLVIII).

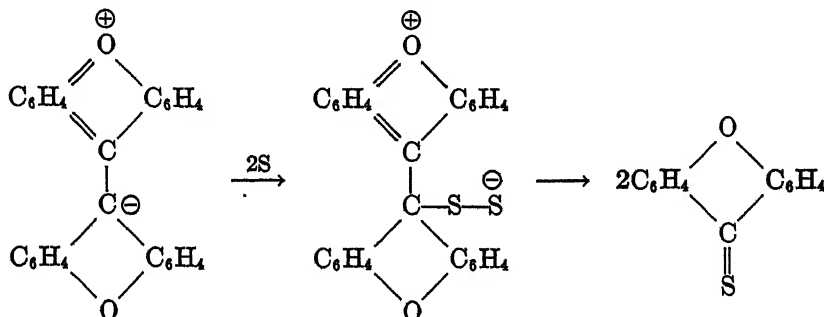


The color changes of the ethylenes (I, XLIX, and L) may be compared (28) with the color changes of certain spiro compounds (8, 10, 16). This phenomenon has also been explained by the formation of betains, as illustrated below:



The above spiro compound forms colorless crystals which become blue on melting. Its solution in hot xylene is deep blue; on cooling it becomes paler and finally colorless (17).

It is possible that the action of sulfur and the action of thionyl chloride on dixanthylene (compare pages 10 and 11) may be explained by the above ethylene-betain theory. That is to say that the action of sulfur on dixanthylene is not the action of sulfur on a true ethylenic compound but proceeds as shown below (25):



The yellow crystals of diflavylene (XXVII) change from yellow to dark red when subjected to pressure (about 100 kg. per 0.01 cm.²) (25) (piezochromism). A similar change of color has been reported in the case of dianthraquinone (L), the yellow crystals becoming bluish green (20).

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BIOCHEMICAL GENETICS

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I. Introduction.....	15
II. The concept of the gene.....	16
III. Hypothesis of gene structure and gene action.....	18
IV. Characters controlled by genes.....	19
A. Psychological characters.....	19
B. Genes affecting chromosome behavior	21
C. Gene stability as a genetic trait	21
D. Morphogenesis.....	22
E. Tyrosine-phenylalanine metabolism in man.....	25
F. The biosynthesis of melanin in mammals.....	28
G. Eye pigments in insects.....	33
H. Anthocyanins and related plant pigments.....	35
I. Disease resistance.....	40
J. Sex phenomena in unicellular organisms.....	43
K. Genes and immunological specificity.....	47
L. Self-sterility in plants.....	52
M. Genes and cancer.....	53
N. Biosynthetic processes in <i>Neurospora</i>	58
O. Miscellaneous specific reactions.....	63
P. Summary of specific gene-controlled reactions.....	65
V. Chemical nature of chromosomes and genes.....	65
A. Isolation and analysis of chromatin.....	65
B. Physical properties of chromosomes.....	69
C. Staining reactions.....	69
D. Ultraviolet-absorption methods.....	70
E. Enzyme digestion.....	70
F. Conclusions.....	70
VI. Gene mutation.....	71
A. Spontaneous changes.....	71
B. Induced changes.....	72
VII. Gene action.....	76
A. Interaction of alleles.....	76
B. Interactions of non-allelic genes.....	79
C. Position effect.....	80
D. Multiple effects of genes (pleiotropism)	81
E. Maternal effects.....	81
F. Genes and protein synthesis.....	82
VIII. Viruses and plasmagenes.....	83
IX. Evolutionary considerations.....	87
X. References.....	88

I. INTRODUCTION

Biochemical genetics may be defined as that branch of biology which seeks to define hereditary units in terms of the chemistry of their structures and of their functions. Mendel most probably wondered about the physical nature

of the unit factors he postulated and about the manner of their action in producing different types of pea plants—for example, those with purple flowers and those with white ones. Certainly, since the rediscovery of his paper in 1900 many others have thought about questions concerning the nature of genes, the equivalents in modern terminology of Mendel's factors.

Biochemical genetics is a field cultivated more by the biologist than by the biochemist. The biologist has been insistently pushed in this direction by advances in genetics, while the biochemist has so far found nothing inherent in his subject that tends so to urge him. History might well have been otherwise, for, as this review attempts to make clear, genes are as much a part of biochemistry as chemistry is of inheritance, development, and function.

The present review attempts to bring together certain facts of genetics and to indicate how these bear on hypotheses concerning gene structure and gene action. For the most part topics have been restricted to those in which facts and interpretations can be expressed in terms of known compounds or reactions about which at least something is understood from a chemical standpoint. Thus, much material often assigned to "physiological genetics" or to "developmental genetics" is omitted. For treatments of material from these viewpoints the reader is referred to Wright (347), Waddington (327, 328), and Goldschmidt (110). For more extensive treatments of "classical genetics," i.e., the mechanics of gene transmission, reference is made to standard textbooks on the subject (66, 268, 276, 308, 327).

II. THE CONCEPT OF THE GENE

The classical geneticist deals with the gene from the standpoint of its behavior in transmission from parent to offspring. In this sense it may be defined as the irreducible unit of inheritance. For the elaboration of a purely formal theory of the type proposed by Mendel, nothing need be assumed as to the physical nature of the gene or its mode of action other than that it somehow causes differentiation of alternative characters in the organism. For example, there are two categories of persons with respect to ability to taste phenylthiourea. To some this is a disagreeably bitter substance not unlike quinine, while to others it is quite tasteless. The mechanical inheritance of this trait can be adequately accounted for by assuming two forms of a factor (gene), one of which conditions the ability of the individual to taste phenylthiourea. The individual receives one or the other of the forms of this gene (alleles) from each of his parents. With respect to individuals there are therefore three combinations of alleles possible, viz., tasters who receive a taster allele from each parent, tasters who receive a taster allele from only one parent, and non-tasters who receive a non-taster allele from each parent. The ability of the second type of person to taste is ascribed to the dominance of the taster form of the gene over its allele.

Actually, of course, it has been known with certainty for some thirty years that genes are carried in chromosomes. Again, from a purely formal point of view it is not a necessity that chromosomes be visible; they could have been predicted from the observations of the inheritance of traits such as taste reac-

tion. This is not to deny that their identification as carriers of genes has been a most powerful factor in advancing the science of genetics.

A view widely held at the present time, therefore, is that genes are units of inheritance, carried in chromosomes which correspond to the linkage groups of the geneticist. The identification of the chromosomes as the carriers of genes is made on the basis of the complete parallelism between the behavior of these bodies as observed under the microscope and the behavior of the genes as inferred from inheritance data. The genes are arranged in a linear order in the chromosomes, and their positions within these linear groups can be determined genetically by using probabilities of recombinations of linked genes as measures of distances. Recombinations of linked genes result from exchange of corresponding segments of homologous chromosomes during the process of chromosome reduction (meiosis). Gene positions (loci) can be independently determined by correlating visible chromosome aberrations with corresponding aberrations in inheritance, e.g., losses of visible segments of chromosomes lead to losses of the genes carried in them.

Genetically it is possible to identify genes only under special circumstances. In the first place, it is not possible to recognize a given gene unless it exists in at least two forms. This can be illustrated by the example of taste reaction to phenylthiourea. If each individual of the human species received a taster allele of this gene from each parent and was therefore homozygous for this form of the gene, there would be no way of inferring that this particular gene existed. Different forms of a gene arise by mutation. As we shall see, gene mutations involve changes of an unknown nature. They may occur spontaneously or their frequency may be artificially increased by treatment with high-energy radiation, such as ultraviolet light, x-rays, and neutrons. A special type of change by which genes can be identified is complete loss.

A second requirement in experimentally identifying a given gene is that its mutation to a new allele, or its loss, results in a detectable change in the organism. Failure to detect a gene-controlled modification may be due to its being quantitatively too small for detection, to there being no suitable qualitative technique for detection of the change, or simply to failure to look for the modification. All of these are experimental limitations. They can be illustrated by an example in nitrogen metabolism. Most mammals, including most races of dogs, excrete purine nitrogen in the form of allantoin. In the Dalmatian coach hound, however, this nitrogen is largely in the form of uric acid. In this respect the Dalmatian differs in one gene from other dogs (322). Knowledge of the existence of this gene is dependent on the detection of the difference in urinary nitrogen. There is no necessary difference in gross appearance between dogs that excrete allantoin and those that excrete uric acid.

In contrast to the possibility that substitution of a given gene has a final effect too small for detection, there is a real possibility that, from the standpoint of the investigator, the effect may be too great. For example, if a given single gene substitution were to prevent cell division, the result would be lethal to any multicellular organism. In certain organisms such characters could be

worked with, and even in man it would be possible to infer the existence of a gene concerned with such a vital phase of early development if sufficient genetic data were available.

The genetic definition of a gene implies sexual reproduction. It is only through segregation and recombination of genes during meiosis and fusion of gametes that the gene exhibits its unitary property. In bacteria, for example, in which cell reproduction is vegetative, there are presumably units functionally homologous with the genes of higher organisms (119, 250a), but there is no means by which these can be identified by the techniques of classical genetics.

It is perhaps worth pointing out that because mutation rates for specific genes are usually low, even following drastic treatment, and because most changes that do occur probably remain undetected experimentally, the majority of the genes of a given organism remain unknown. Even in the vinegar fly *Drosophila melanogaster*, the classical organism of genetics, there are probably at least twenty times as many genes as the few hundred known to geneticists. The detection of such a gene as that for taste reaction to phenylthiourea would not be simple in a vinegar fly.

The biochemical geneticist is concerned with the gene from the standpoint of its chemical structure and its function. He works on the assumption that it is possible to define the gene in chemical terms. Because of limitations in knowledge, such a definition is at present in many respects less satisfactory than that based solely on inheritance. But it can be hoped that the future will see these limitations reduced in number.

It has been seriously argued by Goldschmidt (110) that from a functional standpoint the gene is an artifact, but the evidence for this point of view is not convincing to most biologists. The contrary approach has at least demonstrated its value in stimulating progress. Accordingly, until it is proved otherwise, we are justified in assuming that, however the approach is made, in the end the gene will prove to be an identifiable unit.

If the biochemical geneticist should attain his goal of defining the gene in terms of one or more unique functional properties, many of the experimental limitations discussed above would be eliminated and the scope of the genetic method correspondingly broadened.

III. HYPOTHESIS OF GENE STRUCTURE AND GENE ACTION

As a framework on which to arrange conveniently the varied observations and inferences bearing on the nature of genes and their action it is desirable to have set down a definite summary hypothesis. The presentation of such a hypothesis is undertaken with the realization that alternatives are not only possible but in at least certain respects equally plausible. References are given in connection with later detailed discussions of individual points.

Gene structure—The gene is made up of protein or nucleoprotein. It may correspond to a single giant molecule, or it may be a discrete unit of higher order made up of a group of protein or nucleoprotein molecules, with or without the addition of other substances.

Self-duplication—In order to exist as such, genes obviously must be capable of inducing the formation of exact copies of themselves. The way in which such self-duplication occurs is not known but is presumed to involve some type of model-copy mechanism.

Heterocatalysis—In addition to catalyzing formation of more units like themselves, genes in general have heterocatalytic properties, that is, they catalyze the formation of other substances. The hetero- and auto-catalytic functions are probably essentially similar and consist of imposing specific configurations on protein or other molecules in the final step in their synthesis.

Relation to specific chemical reactions—In determining the specific chemical and perhaps physical configurations of protein molecules, genes directly determine enzyme specificities and thereby control in a primary way enzymatic syntheses and other chemical reactions in the organism.

Gene specificity—Each nucleus of those organisms sufficiently advanced in the evolutionary scale to have nuclei contains many thousands of genes. In diploid nuclei these exist in pairs and in polyploids in groups of higher order. Each of these thousands of gene types has, in general, a unique specificity. This means that a given enzyme will usually have its final specificity set by one and only one gene. The same is true of other unique proteins, for example, those functioning as antigens. ✓

Gene mutation—Through the absorption of energy, which may occur in a number of ways, genes may undergo mutation. If such a mutational change abolishes the autocatalytic property of the gene, the gene is irreversibly lost. On the other hand, if it loses only its heterocatalytic power it remains a gene, but so far as its effect on the organism of which it is a part is concerned it becomes inactive (an "amorph" in the terminology of Muller (327, 347)). Such inactive genes in homozygous forms are of course likely to be deleterious if not actually lethal to the organism. Other types of gene mutations presumed to be possible are those in which the heterocatalytic property is impaired but not destroyed (hypomorphs), those in which the effectiveness of heterocatalysis is increased (hypermorphs), and finally those in which there is a change in one step from one heterocatalytic specificity to another (neomorphs).

IV. CHARACTERS CONTROLLED BY GENES

Almost every aspect of the organism has been shown to be influenced by genetic constitution in one way or another. To indicate the general nature of these relations a series of examples is presented here. In some of these it is not possible at present to see how any simple interpretation can be made in terms of gene action. Nevertheless, they may suggest directions in which future advances can be expected. The sequence of presentation is roughly in the order of decreasing apparent complexity of the gene-character interrelation.

A. Psychological characters

In a number of instances specific behavior patterns are known to be influenced by genes. In man, in whom opportunities are greatest for detecting variations

of this kind, the direct methods of classical genetics cannot ordinarily be used. It is nevertheless possible to gain some information. The so-called twin method is a most useful one in this respect. Identical twins are identical genetically because they develop from a single fertilized egg. Fraternal twins, on the other hand, develop from independently fertilized but simultaneously developing eggs. Intrapair comparisons have been made in both types, with the members of the pairs reared both together and separately (222). Such studies show that differences in behavior can be correlated with differences in genetic constitution. As one might expect, there is no clue as to how such subtle differences are related developmentally to the particular genes that determine them. To illustrate the difficulty of interpreting gene action in such cases Wright (347) has used the example of the web-patterns of spiders. Here interspecific variations are presumably genetically determined. But the manner in which one form of a gene determines that a spider shall construct a web of one pattern while another form results in the construction of a characteristically different web must indeed involve a tortuous chain of events.

Several characteristic abnormalities of the nervous system of man have been referred to single gene modifications. Specific types of feeble-mindedness are almost certainly the result of single gene changes. In one instance it is known that idiocy or imbecility is associated with inability of the individual to oxidize phenylpyruvic acid to its para-hydroxy analogue (98). Further details regarding this situation will be considered later; it suffices here to point out that it is through studies of just such instances as this that the way may be prepared for significant advances in our knowledge of the chemical basis of the functioning of the central nervous system. It is an illustration of how advances come in unexpected ways, and reinforces confidence in the hypothesis stating that, no matter how complex the change may appear to be when one gene allele is substituted for another, the primary modification will ultimately be found to lie in a single chemical reaction.

Another example in which an unpredicted correlation between genetic constitution and response of the central nervous system has been found involved the disease epilepsy. This condition has long been suspected of being hereditary, but its genetic basis appeared not to be simple. Recently Lennox, Gibbs, and Gibbs (174) have found that the brain-wave pattern, revealed by recording fluctuations in potential set up between two electrodes in contact with the head, is characteristic among those predisposed to epileptic seizures as compared with those not so predisposed. The dysrhythmia characteristic of epileptics is inherited as a simple Mendelian dominant character. Fortunately, only about one in twenty persons with this brain-wave pattern ever develops epilepsy. Here again we appear to be a long way from a chemical interpretation, but one can nevertheless say that a first step has been taken.

Taste reaction of individuals to phenylthiourea has already been mentioned. Red-green color blindness is a classical example of a sex-linked recessive trait in man. Since the Y chromosome carries no allele of this gene, all males who carry the mutant allele of this color vision gene in their X chromosomes show

the trait. The female, having two X chromosomes, requires a mutant allele from each parent to show the character. As expected on the basis of mating occurring at random with respect to color blindness, the frequency of the defect in women (about 0.6 per cent) is roughly equal to the square of its frequency in males (about 8.0 per cent). Many other instances of inherited differences in sensory perception probably remain to be discovered. Each individual of a species is likely to be unique in response to the external world because of a unique combination of genes received from his two parents.

B. Genes affecting chromosome behavior

While the physical mechanisms by which genes are transmitted from one generation to the next are remarkably stable in the evolutionary sense, several variations are known. Bacteria have no sexual reproduction as far as we know, and the partitioning of the chromatin at cell division appears to differ significantly from that of higher plants or animals. If gene change is the basis of evolutionary divergence, it follows that these and lesser variations in the mechanics of gene transmission are themselves subject to gene control (65). In fact, there is direct genetic evidence that this is the case. There are now recorded many instances in which abnormalities of one kind or another in cell division are ascribable to specific substitutions of one gene allele for another (65). For example, genes for asynapsis (failure of chromosomes to pair or remain paired during the first meiotic division and leading to irregular chromosome distribution) are known in maize, wheat, rice, barley, cotton, Jimson weed, peas, onion, blue-eyed grass, and the vinegar fly (22, 50, 271, 272). Genically determined failure of cytoplasmic division leading to excessive multiplication of the chromosomes in certain cells is known in corn, wheat, and barley (13, 273). The converse, division of cytoplasm without chromosome division, is also known (12). Possession of divergent spindles at the first meiotic division is a simple recessive trait in maize (50). General "stickiness" of chromosomes leading to breakage, rearrangement, and other aberrations is known to be a recessive character in maize (14).

Cell division is without doubt a complicated process. Yet, if one assumes that each of the gene-controlled deviations mentioned above results from blocking or modification of a single chemical reaction, there would seem to be some hope of finding leads that would bring one closer to a physicochemical interpretation.

C. Gene stability as a genetic trait

Genes vary in their stabilities. So far as can be determined, some of those in the vinegar fly (*Drosophila*) have mutated only once in its thirty-five years history as a laboratory animal (some five hundred generations with hundreds of thousands of individuals under observation in each generation). Others probably exist that have not mutated in a detectable manner during this time. On the other hand, there are many so-called unstable genes in which mutations occur hundreds of times in the development of a single individual (71). That

such differences in mutation rate can be subject to genetic control is shown most clearly by Rhoades (242, 243a). In maize the development of anthocyanin pigment in the aleurone layer (outer layer of the endosperm of the kernel) and in leaves, stems, and other parts of the plant is dependent on a series of non-allelic genes. One of these is the *A,a* pair of alleles. If favorable alleles of all other genes are present, the difference between color and no color is determined by whether a plant (or part of it) has in its cells one or two *A* alleles (i.e., *AA* or *Aa*) or only *a*. The *a* allele is usually very stable. However, in the presence of a dominant allele of the *dotted* gene, which is in an entirely different chromosome pair from the *Aa* alleles, allele *a* undergoes frequent mutation to *A* or other dominant alleles. This is detected by observing patches of colored aleurone or similar colored areas on other parts of the plant. This genic control of specific mutation is significant in several respects. For one thing, it indicates clearly that the *a* allele does not represent merely loss of the *A* gene. Rather, it appears to be a true amorph; it retains the property of autocatalysis but has lost its heterocatalytic function. It is interesting to note that in this instance we know that the gene whose stability is controlled is concerned with some reaction in anthocyanin synthesis but that we have no idea by what means the controlling gene is accomplishing its end result.

D. Morphogenesis

Any treatment beyond mere description of the development of form in organisms cannot be divorced from consideration of the development of function; the first is the physical manifestation of the second. The development of the morphological patterns of higher plants and animals is clearly subject to genetic control, but in contemplating the manner in which this control is brought about we immediately encounter the problem of how cells, tissues, and organs can be differentiated when supposedly all the component cells, being derived by mitotic divisions from the fertilized egg, have identical sets of genes. A blood cell and a nerve cell, for example, differ markedly in both form and function. What is the evidence that they are identical genetically? Why have we rejected views such as those of Roux and of Weismann (339), who held that somatic cell divisions involved *qualitative* divisions of the nuclei by which "determinants" differentially assorted to various parts of the body and gave rise to the many types of cell, tissue, and organ differentiation? Direct proof of the genetic identity of different types of somatic cells is difficult to obtain. We observe that the mitotic divisions of the nuclei accompanying cell division appear to distribute daughter chromosomes equally to daughter cells, but this in no way denies the possibility that genes mutate in a regular fashion and thus account for the process of differentiation. Direct proof would require that differentiated cells be dedifferentiated and shown to be capable of reproducing the whole organism. In higher plants and in some animals this can be approximated. For example, a segment of stem of a grape plant can be rooted, whereupon it gives rise to a new grape plant complete in every respect. But the cutting is known to contain many cells that have remained embryonic. A convincing demon-

stration requires the equivalent of removing the nucleus from a highly specialized cell such as one of nervous tissue, transplanting it to an enucleated egg, and from this reproducing a complete organism. Aside from demonstrating that one descendent nucleus after four nuclear divisions in the fertilized egg (giving sixteen nuclei) can, in the presence of the proper type of cytoplasm, replace the original egg nucleus perfectly in the salamander (281), no such crucial test has been made with higher organisms.

Fortunately there are two reasonably direct ways of showing that differentiation does not involve an irreversible genetic change. One is based on the behavior in development of the protista (single-celled plants and animals). Here, as for example in the ciliate protozoa or certain algae such as *Acetabularia*, described below, we have cellular differentiation carried as far or even further than in higher organisms and with complete reversibility. Certain protozoa with their elaborate system of organelles undergo complete dedifferentiation and redifferentiation at certain times in the life cycle, e.g., during encystment and excystment. Since there is clearly no irreversible genetic change in the process here, there is no need to assume one in the comparable differentiation of individual vegetative cells of the structurally more elaborate metazoa and metaphyta.

A second argument is based on the fact that in the eggs of many metazoa, regions of the cytoplasm are known to be irreversibly determined as to their subsequent fate *before* the division of the egg nucleus (339). It is clear from this observation of experimental embryology that the immediate cause of differentiation lies in the localization of "organ-forming" substances in the cytoplasm and that there is no need to postulate nuclear differences as a basis of ontogenetic divergence of structure and function.

We are therefore returned to the original paradox. Cells of identical genotypes travel many divergent paths in the process of differentiation of the individual organism. Enzyme differences are certainly involved, for it is well known that tissue types differ widely in content of these organic catalysts. This is true also for such substances as hormones, vitamins, and antigens. The agglutinogens that differentiate certain blood types in man, for instance, appear to be confined to the cells of the blood (301). The answer is that pattern differences are evidently set up early in development by apparently trivial circumstances (339). These initially minor differences undergo progressive amplification as development proceeds, leading to what eventually appears to be a most complex integration of form and function. As a specific example of how such a process is thought to work, we can cite the case of the seaweed *Fucus*, as studied by Whitaker (333) and others. The fertilized egg is apparently initially spherically symmetrical. Almost any conceivable asymmetry of the environment upsets this egg symmetry. If it lies on the bottom of a vessel containing the sea water in which it develops, a gradient is set up from top to bottom by differential ease of diffusion of oxygen to the cell and carbon dioxide away from it. In some manner not completely understood but possibly involving a gradient of amount or activity of growth hormone (indole-3-acetic

acid) in the egg, a rhizoid initial (root-like protuberance) is formed on the bottom of the egg. Cell division occurs with the division wall established at right angles to the vertical axis. The initial gradient can be established along any diameter of the egg. Subsequently the axial gradient becomes more and more elaborate (see Child (46) for comprehensive treatment of the rôle of gradients in development and differentiation). The initial minor environmental difference between the upper and lower parts of the egg sets off an orderly train of events that lead eventually to the fully differentiated seaweed. A most significant property of the *Fucus* egg as shown in the work of Whitaker and others is that the initial determination of polarity in the egg can be brought about by different environmental agents, among them: mechanical elongation, stratification of egg contents by centrifugation, temperature gradients across the egg, hydrogen-ion differentials, an electrical potential across the egg, and unilateral differences in illumination with either visible or ultraviolet light.

Although it may appear to be exceedingly difficult to determine in detail how genes exert their influence on a process as complex as differentiation, there are a number of cases in which at least a beginning can be made. One of these is a type of dwarf in the mouse studied by Smith and MacDowell and others (122, 274). This character is differentiated from normal by a single gene. The dwarf allele is recessive. Mice homozygous for this allele attain only about one-sixth the weight of their normal sibs, have defective thyroids, and are entirely incapable of reproduction. Histological examination shows that their pituitary glands are defective in being deficient in a particular type of cell (eosinophiles). If pituitary glands from normal mice are transplanted to dwarfs at the right stage of development, the genetic dwarfs develop into mice that are essentially normal. Their thyroids become normal, they attain an essentially normal size, and the males become fertile. The only defect that remains appears to be that of the anterior lobe of the pituitary. Here, then, the normal allele of the gene concerned evidently has something to do with the production of one or more of the growth hormones normally elaborated by the anterior lobe of the pituitary gland, and known to influence developmental processes other than growth.

Somewhat similar situations are known in the case of dwarfness in maize (325a), in biennial *versus* annual growth habit in certain plants (206), and in a number of other organisms (110, 221, 328).

Genes evidently determine the final potentialities of differentiation in a given organism. The initiation of the orderly process may be through the environment, but whether or not a given reaction in the time-space sequence can take place is gene controlled. If a given gene is present in a cell, the presence of an enzyme with a corresponding specificity is dependent on the antecedent steps in the developmental sequence. If these are right, it will be present, otherwise not. But if the gene is absent or defective, the enzyme cannot be formed regardless of what has gone before. Wright (348) has ably summarized this genetic point of view regarding developmental processes, but recognizes that its application as a whole in individual instances is not simple. What is needed

is a resolution of the problem into simpler components. This of course is constantly being done—at the morphological level by students of organizer phenomena, at a physiological level by those responsible for the development of gradient theories, and finally at a chemical level by the biochemist. The genetic approach has been much neglected. The possibilities of systematically studying changes brought about in morphogenetic processes by substitutions of single genes are virtually unlimited. If each change is really referable to a change in a single primary reaction, then the method should be a most powerful one.

E. Tyrosine-phenylalanine metabolism in man

During the present century several inherited errors having to do with the metabolism of the amino acids tyrosine and phenylalanine have been described in man. One of these in particular is a classic of biochemical genetics, because it represents the first instance in which a particular gene was related to a specific and known biochemical reaction. It is the disease known as alcaptonuria. One characteristic symptom of this metabolic deviation, blackening of the urine on exposure to air, was recorded three hundred sixty years ago (101). Eighty-seven years ago Bodecker isolated the substance responsible for this discoloration of the urine and found it to be 2,5-dihydroxyphenylacetic acid, also known under the names alcapton and homogentisic acid (101). Shortly after the rediscovery of Mendel's paper in 1900, Bateson and Punnett pointed out that alcaptonuria behaved in inheritance like a simple Mendelian recessive trait (10). In the first edition of *Inborn Errors of Metabolism*, published in 1909, Garrod (101) summarized the accumulated information as to the nature and inheritance of alcaptonuria. Five years later Gross (101) found that the blood serum of normal individuals contains an enzyme capable of catalyzing the breakdown of homogentisic acid. This enzyme is not found in the sera of alcaptonurics. Thus, thirty-one years ago, it was clearly established that a single gene substitution results in the absence or inactivity of a specific enzyme and that this in turn leads to the failure of a particular biochemical reaction. It is interesting that no clearer example exists today.

From a historical standpoint it is a curious fact that until recent years alcaptonuria has played almost no part in the development of theories of gene action. Garrod's book (referred to above) and its second edition, published in 1923 (101), treated alcaptonuria in detail from both the biochemical and the genetic points of view. This book, which also contains accounts of other inherited metabolic abnormalities in man, should be credited as representing the beginning of biochemical genetics, but unfortunately, until its significance was pointed out recently, particularly by Haldane, it has remained practically unknown to geneticists. The biochemists, it should be said, were less guilty of neglect, but they apparently were not prepared to appreciate fully the genetic implications.

Because the studies on alcaptonuria, many of which were carried out by Garrod himself, are of interest both historically and currently in illustrating methods of biochemical genetics, they are well worth consideration in some detail. (See Garrod (101) for references and additional details.) Normally, 2,5-

dihydroxyphenylacetic acid is broken down, probably by way of acetoacetic acid, to carbon dioxide and water. In alcaptonurics this breakdown is blocked. It would therefore be expected that normal precursors of homogentisic acid, or compounds convertible to such precursors by reactions carried out in the organism, would lead to increased excretion of this intermediate in the breakdown process, but would be completely metabolized by normal individuals. Following this reasoning a series of compounds has been fed to alcaptonurics and to normal controls. As expected, homogentisic acid ingested by alcaptonurics is excreted, but is broken down by normal persons. Both phenylalanine and tyrosine lead to increased homogentisic acid in the urine of alcaptonurics, but not in normal individuals, and are therefore judged to be initial precursors. p-Hydroxyphenylpyruvic acid is converted to homogentisic acid, while its lactic acid analogue is not. Because p-hydroxyphenylacetic acid is not converted to homogentisic acid, whereas 2,5-dihydroxyphenylpyruvic acid is, it is thought that the oxidation of the aromatic carbon atoms must precede that of the side chain. Gentisic acid (2,5-dihydroxybenzoic acid) is excreted by sufferers from alcaptonuria but is metabolized by normal persons. Benzoic acid oxidized at other positions in the ring is metabolized by both normals and alcaptonurics. It thus appears that the specific disability induced by the gene substitution is that of failure to disrupt the ring structure in 2,5-dihydroxyphenyl compounds.

A second inherited abnormality of phenylalanine-tyrosine metabolism in man is responsible for the condition known as albinism. Here melanin formation is greatly reduced. Again, our knowledge of this trait was brought together by Garrod in 1923 (401). In at least most instances albinism behaves as a simple Mendelian recessive character. Unfortunately, the chemical transformations by which melanin is formed are not completely understood (97, 238). Apparently tyrosine is oxidized to 3,4-dihydroxyphenylalanine as a first step. There is present in most mammals an aerobic oxidase which is concerned with the further oxidation of this compound. In the biological literature 3,4-dihydroxyphenylalanine is often known as dopa and the enzyme catalyzing its oxidation as dopa oxidase. Whatever may be the exact mechanism of melanin formation from 3,4-dihydroxyphenylalanine, some one of its steps is evidently interfered with when the recessive allele of the albino gene replaces its normal allele. It is interesting that albino mutant types, presumably biochemically similar to that in man, are known in many other mammals and in a number of animals of other groups. In fact, elaborate genetic analyses of melanin formation have been made in a number of these. These studies are summarized in the following section of this review.

Since the publication of the second edition of *Inborn Errors of Metabolism*, two additional defects in phenylalanine-tyrosine metabolism have been described in man. One of these, failure to oxidize phenylpyruvic acid—a condition known as phenylketonuria—has already been mentioned. This condition was first discovered by Fölling (98) and has since been studied by Penrose and others (130, 231). Like alcaptonuria and albinism, this condition is inherited as a simple recessive character. As has already been pointed out, a most unfortunate

and apparently invariable symptom of phenylketonuria is idiocy or imbecility. If phenylpyruvic acid is fed to a phenylketonuric under conditions in which it would be oxidized by a normal person, an increased phenylpyruvic acid content of the blood and urine is observed. The same is true if phenylalanine is fed. No difficulty is encountered in the oxidation of tyrosine by persons suffering from this disease. The assumption is that the normal allele of the gene for phenylketonuria is concerned with the reaction by which phenylpyruvic acid is oxidized to its para-hydroxy analogue.

Phenylalanine is usually assumed to be directly oxidized to tyrosine. It is possible that this oxidation is sufficiently similar to that of phenylpyruvic acid to its para-hydroxy analogue to be catalyzed by the same enzyme and therefore to be controlled by the same gene. If these assumptions were correct, one would expect a phenylketonuric to be unable to make tyrosine from phenylalanine and consequently to require tyrosine as an indispensable component of the diet. An alternative mechanism for the conversion of phenylalanine to tyrosine (suggested by Dr. H. K. Mitchell, personal communication) involves conversion of phenylalanine to its keto acid analogue, oxidation of this in the para-position, followed by reamination to tyrosine. In this case, too, phenylketonurics would require dietary tyrosine. Experimental evidence on the dispensability of tyrosine in the diets of phenylketonurics might therefore be most illuminating. These considerations suggest that the reason mammals have not lost the ability to oxidize phenylalanine to tyrosine during the course of their evolutionary specialization may be that the reaction by which this is accomplished plays a second rôle which, if not exactly that indicated, may still confer a strong selective advantage. This type of relation may well be of general importance and may in many instances account for the fact that from "indispensable" dietary compounds, organisms retain the power to make those "dispensable" ones that occur regularly in the diet in amounts sufficient to meet the needs of the organisms.

Medes (205) has reported studies on a man who was apparently unable to carry out the rather curious oxidation of *p*-hydroxyphenylpyruvic acid to its 2,5-dihydroxy analogue. Ingested precursors of *p*-hydroxyphenylpyruvic acid led to increased excretion of this substance in the urine in a manner analogous to their action in alcaptonuria. From a scientific standpoint it is unfortunate that this disease, known as tyrosinosis, has been reported in only a single individual. We therefore have no way of knowing about its inheritance, although by analogy we might expect it to result from homozygosis for a single mutant gene.

A summary of phenylalanine-tyrosine metabolism in man is given in figure 1. The supposed interrelations of the known and suspected naturally occurring relevant compounds are shown here, based on an interpretation by Haldane (130). This is by no means the only possible scheme so far as details are concerned. The facts and interpretations on which this scheme is based illustrate several points of significance to biochemical genetics. Gene action can be interpreted in terms of a one-gene-one-reaction hypothesis. In one case a specific enzyme is known to intervene between gene and character, and in the others a

similar assumption is compatible with the facts. The use of specific genetic blocks in working out the course of metabolism is also well illustrated. Not only can we say that the existence of the four defective types helps us to understand the system of reactions but, if additional types were available, the course of the process could be described in more detail and with more confidence.

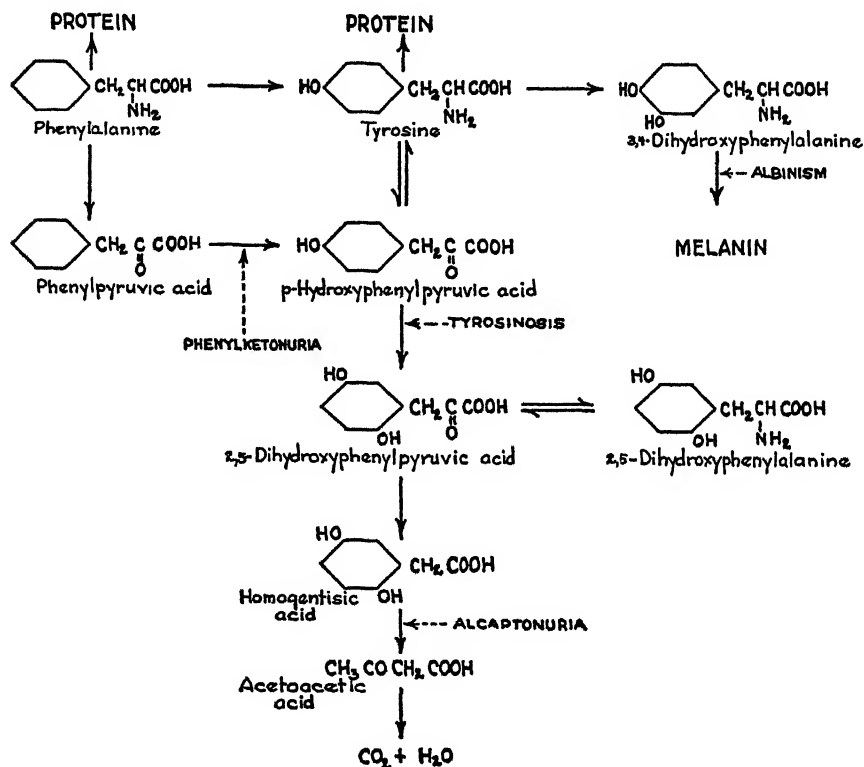


FIG. 1. Scheme of phenylalanine-tyrosine metabolism in man (based on interpretation given by Haldane (130)).

F. The biosynthesis of melanin in mammals

The pigments responsible for the coat colors of mammals are melanins. The familiar variations in such animals as chickens, horses, cattle, and man (skin and hair color) are for the most part genetically conditioned and result from both quantitative and qualitative modifications of the melanin pigments. The actual pigments are largely confined to special ameboid cells known as melanophores. In the birds and amphibia these are known to originate during embryonic stages in the neural crest, a tissue lying just under the ectoderm and over the nerve tube. The evidence for mammals is less conclusive (348), but there appears to be no reason for doubting that here, too, potential pigment cells arise in the neural crest. During development the melanophores migrate from the

neural crest to various parts of the body. Their path of movement is mainly just beneath the ectoderm. In the fishes and amphibia and in general in the case of "skin" pigmentation the melanophores migrate to their definitive positions and there produce pigment in the form of intracellular granules. In hairs and feathers, on the other hand, the melanophores migrate to the hair and feather follicles, where they physically discharge pigment granules into the developing hairs or feathers. In the fowl, where this process has been studied in detail by Dorris, Eastlick, Willier, Rawles, and others (see review of Du Shane (78) for references and additional details), it is known that the life span of pigment cells, the type of pigment they produce, their rhythmic response to local conditions as seen in the barred rock, for example, and other characteristics are subject to their own genetic constitutions.

Essentially similar conditions are found in the amphibia, where Du Shane (77), Twitty (325), and others have shown that the migration of pigment cells, their distribution patterns, and their capacities to produce pigment are subject to genetic control. It is known in these animals that whether or not a melanophore actually produces pigment depends on the constitution of the overlying ectoderm. Thus, the white axolotl has melanophores which ordinarily produce little or no pigment but which can be induced to do so by experimentally placing them in the proper relation to an ectoderm of the proper genotype (77). In culturing salamander melanophores *in vitro* Twitty (325) has observed a negative correlation between cell motility and pigment formation. This relation may play a significant part in pigment pattern formation in these animals.

Of the mammals the guinea pig is perhaps best known from the standpoint of the genetics of coat color. This is largely due to the efforts of Sewall Wright, who has contributed to our knowledge of the subject over a period of some thirty years. (For details and references beyond those given here the reader is referred to Wright's general reviews (346, 347, 348).)

There appear to be two primary pigments in the guinea pig, both melanins. One of these, the xanthic pigment, is characteristic of red and yellow coat color. The other, known as melanic pigment, is found in black, sepia, and brown animals. Because of general limitations in our knowledge of the chemical constitution and behavior of melanin pigments, it is an open question whether or not these two types of pigment differ in chemical structure. Their absorption spectra appear to differ significantly (7, 109), but this may possibly represent only a difference in colloidal state.

In control of qualitative and quantitative aspects of pigment formation, the distribution of pigmented hairs on the animal, and the distribution of pigments within individual hairs, there are seven known major genes. The albino series of alleles of which five members are known is evidently homologous with the albino genes known in other mammals. This series is concerned with the quantity of both pigments and evidently concerns some process common to the formation of both xanthic and melanic pigments. The normal allele of this gene, *C*, is completely dominant to other known alleles. Intermediate members, on the other hand, show incomplete dominance over less active alleles. The

lowest member of the series, c^* , is essentially an amorph; animals homozygous for it are devoid of pigment under ordinary conditions. Russell's tests for enzymes (254) in frozen skin sections using 3,4-dihydroxyphenylalanine as a chromogen were negative for albino animals regardless of the residual genotype. It is suggested by Wright that the C series of alleles is concerned with the quantity or activity of the two enzymes concerned with the two pigment reactions (figure 2), although he recognizes the possibility of alternative interpretations.

The P gene appears to be concerned solely with the melanic pigment system. It is completely dominant. In ff animals no sepia pigment is formed in the absence of a P allele, i.e., in pp animals.

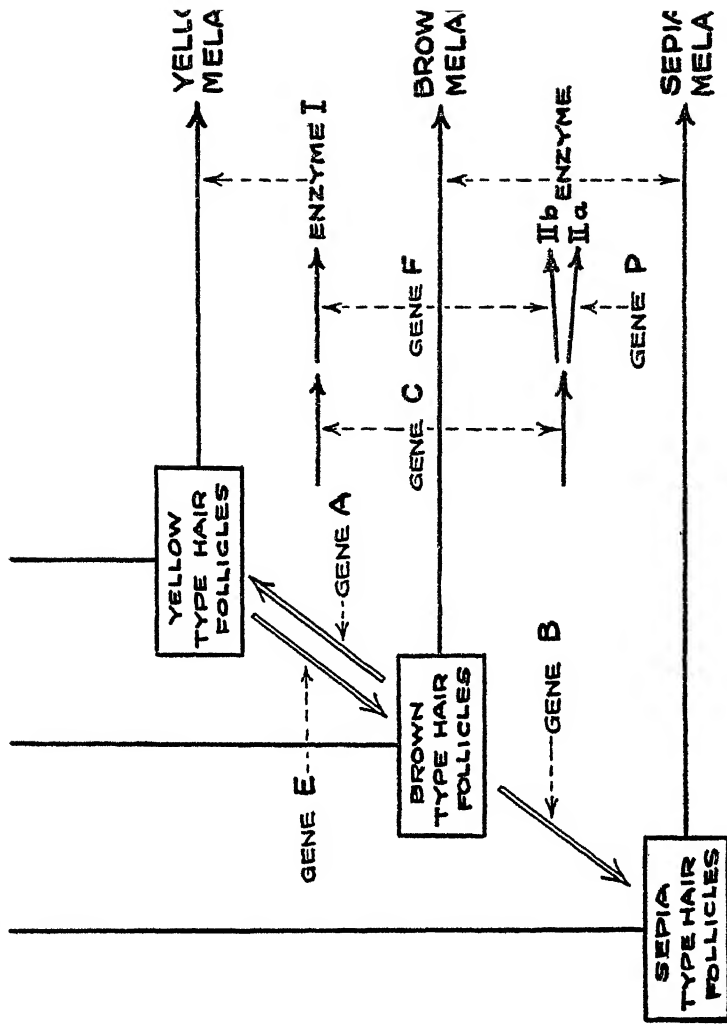
While the Ff alleles seem to be concerned primarily with the xanthic pigments, F can partially replace P under certain conditions. In pp animals with an active allele at the C locus, some sepia pigment is formed in FF and Ff animals but none is detectable in ff individuals. Wright (350) therefore suggests that P and F act on partially equivalent steps in the formation of melanic enzyme (enzyme IIa or IIb of figure 2). With respect to its relation to xanthic pigment, F is complementary to C . Both must be present for the full amount of yellow pigment to be formed. If C is replaced by a completely inactive allele, no pigment is produced regardless of the condition of F . If F is replaced by f in the presence of C , on the other hand, yellow pigment is reduced but not entirely abolished. The f allele may be acting as a hypomorph rather than as an amorph.

Genes B and E are interpreted by Wright (348, 350) as having to do with the differentiation of hair follicles. These are postulated to be of three types, as indicated in figure 2, and they are assumed to determine the course of melanin formation along three corresponding pathways. Gene E determines that the follicles will be of either of the two melanic types, while gene B determines that a melanic-type hair follicle will be of the sepia rather than the brown subtype characteristic of $E\ bb$ animals.

The gene A (for agouti pattern) is concerned with a blocking of melanic pigment differentiation in local regions of individual hairs. Instead of black or sepia there appears red or yellow pigment in a characteristically localized segment of each hair.

The two types of pigment, xanthic and melanic, appear to be competitive in their formation, as though they were derived from a common precursor limited in amount. Thus, black pigment is not simply superimposed on a normal quantity of yellow in a black animal but is laid down at the expense of some of the yellow pigment.

There is an incompletely recessive mutant allele, s , which in contrast to its normal allele results in pigment appearing in sharply delimited areas of the body. In the main it has little or nothing to do with the type of pigment formed but somehow has to do with its distribution (351). The mechanism by which one area of skin differs from another is not known, but it is clear that the differential is established during embryonic development of cells. It is suggested (348) that spotting may be concerned with locally arrested pigment cell migration or with a threshold condition in embryonic cells that determines whether pigment cells persist or not.



Scheme of melanin synthesis in the guinea pig (based on work and interpretation of Wright (346, 347, 348, 350)). It is not certain that 3,4-dihydroxyphenylalanine is the common pigment precursor.

Wright (348) has shown that the formation of melanic pigments is subject to temperature modification. If the skin temperature is lowered, albino animals develop some "sootiness," particularly in the extremities, in which the mechanism for control of body temperature is relatively less effective. A similar situation has long been known in the Himalayan rabbit, in which at a low temperature the ears, feet, tail, and snout develop black pigment. Other parts of the body are capable of developing pigment if the skin temperature is kept below 33°C. for a sufficient time. In this and other genetic types of the rabbit Danneel and Schaumann have presented evidence indicating that melanin formation involves a sequence of at least three reactions, the first capable of being suppressed by x-rays and involving the formation of "lipochochondria," the second involving aerobic formation of a specific enzyme similar to, if not identical with, dopa oxidase (temperature-sensitive in the Himalayan type and absent in the true albino), and the third, a process of pigment formation requiring oxygen and inhibited by hydrogen cyanide (review, 64).

Siamese and Burmese cats are genetically analogous to the Himalayan rabbit and are physiologically similar in being more heavily pigmented in exposed parts subject to lower temperatures during pigment development (316).

Methods for the quantitative estimation of pigments in the guinea pig have been developed by Russell (252) and Heidenthal (131), following earlier work by Durham, Gortner, Einsele, and others (348). Working with Wright's genetically known material, these investigators have made extensive measurements of the pigments present; particularly in relation to substitution of various alleles at the *C* locus. On the basis of these measurements and his own genetic analysis, Wright has elaborated a formal scheme which, when qualified with suitable rate constants and in other ways, gives remarkable quantitative agreement with observed pigment levels. A much reduced version of this scheme is given in figure 2. In connection with the development of this interpretive scheme Wright has done much pioneer work in the development of theories of gene action. For anyone interested in the details of this development it is necessary to consult the original papers (344, 346, 347, 348).

The interpretation on which figure 2 is based assumes that a single gene may be concerned with the production of two different enzymes. This, however, has not been demonstrated beyond doubt. It seems possible that enzymes I and II may be essentially similar and that the difference in the end products may depend on the conditions under which they act, e.g., type of hair follicle in which pigment is produced. More data are needed before the scheme depicted can be interpreted in detail in terms of enzymes and specific chemical reactions.

While the difficulty of working with melanin-forming reactions *in vitro* has been a serious drawback in the analysis of the guinea pig coat colors, it is nevertheless remarkable how far biochemical genetics has been able to go in this case. It is perhaps worth indicating that it is easy to underestimate the general significance of studies of this kind. Embryologists have often said, "We are not interested in the color of the hair of a guinea pig—this is trivial; what we

want to know is how and why does the eye differentiate?" It is true that the pigmentation of the hair is not of vital importance to the welfare of the guinea pig—but it is not trivial. It is precisely because its presence is not of vital importance to the animal that we can learn so much about its formation and differentiation. Gene substitutions which alter the system are not lethal but leave the organism intact for study. Furthermore, there is no reason whatever to suppose that genes that concern non-vital phenomena differ in any significant way in their manner of action from those related to processes with which the organism cannot dispense. In fact, as we shall see, there is positive evidence that gene action is fundamentally the same in both types of processes.

G. Eye pigments in insects

Without doubt the most complete analysis of any pigment system from a genetic standpoint is that of eye colors in the vinegar fly *Drosophila melanogaster* (32, 216). Mutations of some twenty-five different genes are known to modify the final eye color from the deep red of the wild-type fly. It has been deduced from observations on interactions of different mutant types that there are two independent pigments (198, 343). One of these, a brown pigment, can be removed by gene substitution at any one of four gene loci, leaving only the red component. The resulting eye color is bright red. The red pigment can be removed by replacing the normal allele of the gene *bw* by an inactive allele. This leaves only the brown component. Both pigments can be blocked by a single gene change at the white locus. The physiological interpretation of these facts is that there are two reaction chains leading to brown and red pigment components, but having at some stage some step in common (198, 343). It is this common step that is dependent on the normal allele of the white gene.

The chemical nature of the two pigment components has been studied by Becker (23), by Clancy (49), and by Ephrussi and Herold (90). The brown pigment, known under Becker's name "ommatin," is widely distributed in insects and appears to be a compound of low molecular weight. As will be indicated below, it seems always to be dependent for its formation on the intervention of tryptophan derivatives. It is not soluble in water or in the usual organic solvents, but is soluble in acidified alcohols. It is a redox and pH indicator, is probably bound to a protein *in vivo*, can be benzoylated, and shows a color change in the presence of mineral acids. The red pigment is similar in being of low molecular weight, in probably forming a protein complex, and in showing pH and oxidation-reduction color changes. It differs in solubility, in being independent of the tryptophan reactions, and in not being benzoylated. By the use of absolute methyl alcohol acidified with 1 per cent of dry hydrogen chloride as a solvent for brown pigment and 30 per cent ethyl alcohol taken to pH 2.0 with hydrogen chloride for the red pigment, accurate methods for the quantitative measurement of eye pigments have been developed (49, 90).

The development of the brown pigment component has been shown by Sturtevant and by Ephrussi and Beadle to be dependent on substances capable of diffusing from one part of the body to another (89). These hormone-like sub-

stances have been identified through the efforts of several workers as tryptophan derivatives. Their postulated relations to the brown pigment and to the gene-controlled reactions leading to its formation are indicated schematically in figure 3. Dietary tryptophan is the fly's initial precursor of the two postulated hormones (315). This is converted to alpha-oxytryptophan through a reaction controlled by the vermilion gene (37). A further oxidation to kynurenine occurs. (The Kotake formula of kynurenine has recently been shown to be

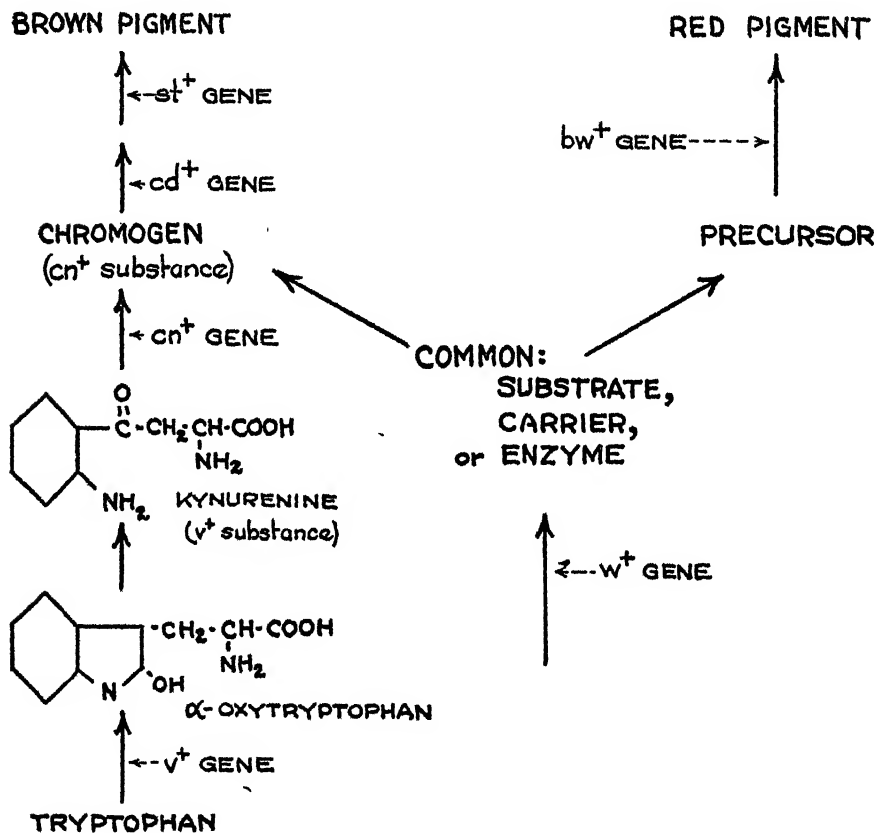


FIG. 3. Representation of insect eye-pigment development

incorrect by Butenandt *et al.* (38).) This is the so-called v⁺ substance of Ephrussi and Beadle. This is still further oxidized to the cn⁺ substance, which Kikkawa (149) believes to be the chromogen of the brown pigment. The transformation of kynurenine to cn⁺ substance is subject to the action of the normal allele of the cinnabar gene. Its recessive allele, when homozygous, results in failure of this action.

Other gene-controlled reactions must contribute to the sequence leading to brown pigment, for it is known that this pigment fails if either the scarlet or the cardinal gene mutates to an amorph. These genes must intervene after the

function of cn^+ substance or in a parallel reaction chain, because flies of both mutant types produce both kynurenine and cn^+ hormone.

Little is known about the reactions by which the red pigment is produced, except that it is dependent on the activity of the brown gene. Since the synthesis of both pigments can be blocked by a change at the white locus, it is essential to postulate a common step in the formation of the two pigments. The nature of this common step remains unknown, however.

The manner of action of the eye-color genes not shown in figure 3 is not known. Some of them influence the quantities of one or both pigment components. In the case of *sepia* there is apparently a qualitative change of the red pigment (198), but the nature of the modification is not clear. The suppressor-of-vermilion mutant type involves a change in a sex-linked gene. In the homozygous condition this gene somehow operates to restore brown pigment to flies which are homozygous for the vermilion gene and which normally lack brown pigment (18).

Somewhat similar genetic and biochemical antecedents to eye-pigment deposition are known in several other insects. Thus, in the silkworm *Bombyx mori* the white-I mutant type corresponds physiologically to the cinnabar type of *Drosophila* (149). The mutation responsible for blocking the transformation of kynurenine to + chromogen (cn^+ substance) presumably involves a gene homologous to the cinnabar gene of *Drosophila*. The ivory mutant type of the parasitic wasp *Habrobracon* likewise corresponds in both respects to the cinnabar gene of *Drosophila* (16). The meal moth *Ephestia* has black eyes. A red-eyed mutant type found by Kühn and known to be a monogenic recessive was first shown by Caspari (40) to be differentiated by the absence of a diffusible substance, the so-called a^+ hormone. This is now known to be kynurenine (37). The *Ephestia a* allele appears to represent a mutation parallel to that giving rise to the vermilion allele in *Drosophila*. Historically, work on the a^+ hormone of *Ephestia* antedated that on the hormone of the corresponding eye color of *Drosophila*.

It is interesting to observe that as our knowledge of eye pigments of insects and their genetic control has increased, hypotheses concerned with the manner of gene action have become increasingly specific and in certain respects simpler than their forerunners. The facts are certainly not incompatible with the thesis that to every gene it is possible to assign one primary action and that, conversely, every enzymatically controlled chemical transformation is under the immediate supervision of one gene, and in general only one.

H. Anthocyanins and related plant pigments

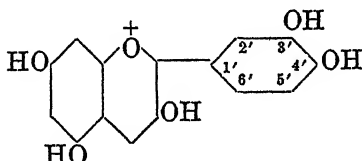
Most of the water-soluble red, blue, and yellow pigments found in flowers and other parts of plants are anthocyanins or related compounds. More than thirty years ago Wheldale (who later wrote under the name Onslow) appreciated that the intraspecific variation with regard to these pigments offered an unusual opportunity to relate genes to the specific structure of the pigments dependent on their activity (332). With subsequent advances in our knowledge

of the chemistry of these pigments due to the activities of Willstätter, Karrer, the Robinsons, and others, it has become possible for biochemical geneticists to make really substantial progress along the lines established by Wheldale.

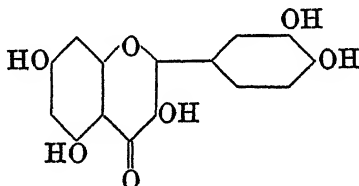
Flowers owe their colors to five types of pigments:

(a) *The carotenoids*, not sap soluble, and usually confined to plastids.

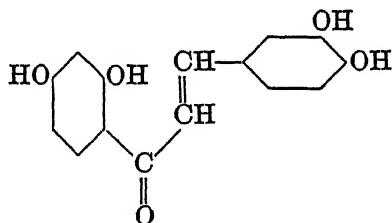
(b) *Anthocyanins* (anthocyanidin glycosides): Cyanidin is a common anthocyanidin type and has the structure:



(c) *Anthoxanthins and derivatives*: Quercetin is a commonly occurring anthoxanthin. It has the structure:



(d) *Chalcones*, probably usually as glycosides: Butein belongs to this group of pigments. Its structure is



(e) *Flavocyanins*: These are water-soluble, anthocyanin-like, yellow pigments, the structures of which have not been completely established. Nudicaulin, found in species of the poppy (*Papaver*), is an example (93, 237).

Although several of these pigments have been synthesized *in vitro*, the mechanism by which plants make them is not definitely known. Perhaps the most widely accepted theory is that of Robinson (170, 248), which postulates that both anthocyanins and anthoxanthins have a common origin in the union through aldol condensations and dehydrations of two hexose units and one triose unit. The hypothetical intermediate shown in figure 4, and here designated as the Robinson precursor, is believed to result. From this, cyanidin can be derived through oxidation at carbon atom 1, dehydration between carbon atoms 2 and 3, and ring closure. On the other hand, oxidation at 1 and 3 or at 2 and 3, followed by ring closure, would give quercetin. It is evident that the

Robinson theory can readily be extended to include the formation of chalcones. Through reduction at position 3', cyanidin gives rise to pelargonidin, while oxidation at 5' gives delphinidin (figure 4). Analogous modifications of quercetin are known.

So-called leucoanthocyanins occur naturally and have been postulated by Robinson (171, 248) to act in special cases as anthocyanin precursors, for example, in autumn coloration of leaves. A commonly occurring leucoanthocyanin, the structure of which is not known, gives rise to cyanidin on treatment with hydrochloric or sulfuric acid. Bancroft and Rutzler (8) ascribe a more general rôle to leucoanthocyanins in anthocyanin biosynthesis, but their evidence is not wholly convincing. It seems clear that further investigation is

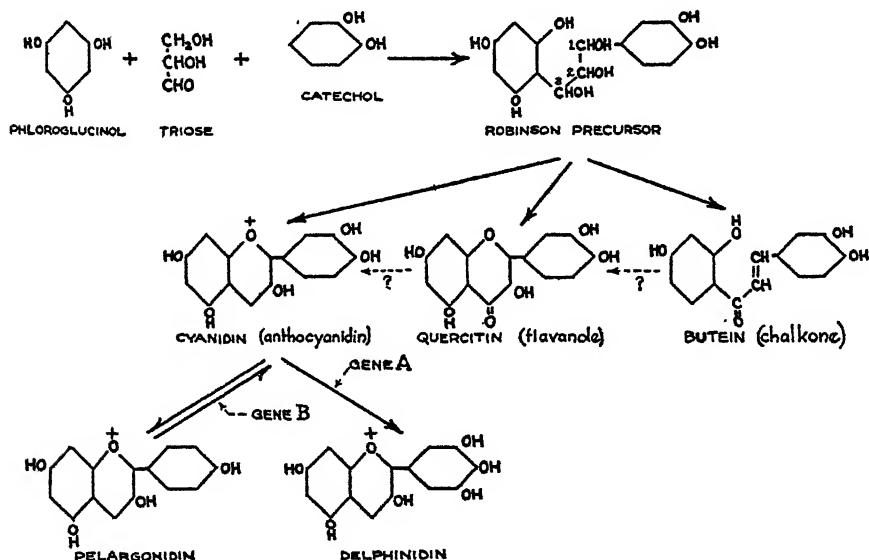


FIG. 4. Interpretation of development of anthocyanin and related pigments (based on Robinson (248) and Lawrence and Price (170)).

necessary before the part played by the leucoanthocyanins can be precisely defined.

Flower pigments are modified genetically in several important ways. In the first place, their presence is determined by the genotype. Thus in the Chinese aster *Cheiranthus cheiri* the dominant gene *Y* is necessary for the appearance of a carotenoid pigment in the petal plastids (265). The water-soluble pigments may or may not be superimposed on this. In a similar way a dominant gene is known to be necessary for the presence of an anthoxanthin in the primrose *Primula acaulis* (170). In the sweet pea *Lathyrus odoratus* complementary dominant genes are necessary for anthoxanthins, i.e., if either of these is present in homozygous recessive form, no anthoxanthin is formed. The presence of anthoxanthin is not dependent on that of anthocyanin. On the assumption that these two genes sponsor different reactions, this would imply that at least

two reactions intervene between the common precursor and anthoxanthin, a conclusion that does not seem unreasonable from a chemical point of view.

The chalcone butein has been isolated from the petals of *Dahlia variabilis*, where its presence is dependent on the dominant allele of a specific gene (236). It has likewise been isolated from *Coreopsis species* and from *Cosmos sulphureus*, where it occurs as the glycoside coreopsin (103, 104, 105, 106). The chalcones as flower pigments have only recently been investigated. Whether they show genetically controlled chemical variations analogous to those of the anthocyanins and anthoxanthins remains to be determined.

In several species the presence or absence of anthocyanins is genetically determined and seems to be qualitatively independent of the presence or absence of anthoxanthins and chalcones. In *Cheiranthus*, *Lathyrus*, and stocks (*Matthiola incana*) the presence of anthocyanins depends on two complementary factors. In flax (*Linum usitatissimum*) three complementary factors are known to be necessary for anthocyanin formation. If in these cases the genes concerned affect only anthocyanin synthesis, they must concern reactions by which the common precursor (figure 4) is transformed into the final pigments. This appears to be the situation in *Lathyrus* at least (21). In both the Japanese morning glory (*Pharbitis nil*) and the snapdragon (*Antirrhinum*), on the other hand, genes are known which simultaneously control both anthocyanin and anthoxanthin pigments (21). If in either case the recessive allele is substituted, neither type of pigment is formed. These genes may control some reaction by which a common precursor is synthesized or control the specificity of an enzyme common to the synthesis of both anthocyanin and anthoxanthin.

In the above-mentioned instances, presence of pigment is genetically dominant to its absence. Presumably the active forms of the genes are necessary. There are so-called dominant whites known in a number of forms, for example, in *Pharbitis nil* (170). The manner of action of such dominant "inhibitors" of specific chemical reactions is not known, but it is possible to devise plausible hypotheses to account for it.

If anthocyanin pigments are produced, they may be genetically modified in various ways. One of these ways is in the degree of oxidation of the prime ring. Types in which hydroxyl groups occur at positions 3' and 5' in addition to those always present at 4' (delphinidin types) are usually dominant to those oxidized at positions 3' and 4' (cyanidin types) and to those oxidized only at 4' (pelargonidin types). Cyanidin types are usually dominant to pelargonidin types. Beale (20) has tabulated the predominant direction of mutation for the wild type in a number of species where this is known. This turns out to be from dominant to recessive, i.e., from more oxidized wild-type pigments to less oxidized mutant-type pigments. In two genera, *Lathyrus* and *Streptocarpus*, the interaction of two pairs of alleles concerned with these oxidative differences is known. It is of the following type:

<i>Genes present</i>	<i>Pigment type</i>
A B	Delphinidin
A b	Delphinidin

This type of interaction is not consistent with any simple scheme in which the pigments are synthesized in series from less oxidized to more oxidized, or the reverse. It does, however, agree with the scheme of figure 4 on the assumption that gene *A* conditions the presence of an oxidase specific for the cyanidin configuration, while gene *B* determines the presence of an oxidase specific for that of pelargonidin. *A* leads to oxidation at the 5'-position, while *B* does so at the 3'-position. If the cyanidin type is the first formed, as the evidence seems to indicate, it is necessary to suppose that the oxidase corresponding to gene *B* acts to reverse reduction at position 3', otherwise postulated to occur spontaneously.

Glycoside formation is known to be genetically controlled in two instances, *Verbena* and *Streptocarpus* (170). The situation in *Verbena* is simpler; the 3,5-dimonoside type differs genetically from the 3-monoside type of anthocyanin.

Methylation of hydroxyl groups 3' or 3' and 5' is possible. This is dependent on previous oxidation at these positions and is related to glycosidal type as well. The genetic basis of differences in methylation appears not to be simple in *Streptocarpus*, where it has been studied (170, 172).

Another source of variability of flower color lies in the phenomenon of "copigmentation." Anthoxanthins, tannins, and possibly other compounds may form weak addition complexes with anthocyanin pigments. Such complexes have their color deepened more than would be expected on a simple additive basis. Insofar as presence as well as types of anthoxanthins are dependent on the genotype, copigmented types may be differentiated from those not so modified by a single gene change (170).

Since anthocyanins are pH indicators, variations in the hydrogen ion of the cell sap in which they are found would be expected to modify their color spectra. Such variation is known in at least half a dozen species (170), and is further known to be dependent on the genotype. In all cases more acid petal-cell sap is dominant to the less acid type. The difference is of the order of one-half to one pH unit, and it is interesting that the difference is strictly localized in the petals.

While the data are not yet sufficient to enable one to formulate an interpretation that it is beyond question, they do indicate that relations somewhat similar to those portrayed in figure 4 must exist. The scheme may have to be modified in many details. Furthermore, it is not certain that one simple scheme will apply to all plants. For example, in maize the gene pair *Aa* (see page 22), in the presence of suitable alleles of other genes, differentiates between anthocyanidin (cyanidin-3-glucoside) and corresponding anthoxanthin (quercetin-3-glucoside). Genotypes carrying *A* (*AA* and *Aa*) have the cyanidin derivative in the stems, leaves, husks, and other parts of the plant, while the homozygous recessive form (*aa*) contains the corresponding quercetin derivative (257). This situation has been used as a basis for the argument that the anthocyanin nucleus is derived from that of anthoxanthin through reduction (8, 257). Lawrence and Price (170) and the Robinsons (249), however, are not convinced by this argument.

The hypothesis of a common precursor for the three related water-soluble pigments derives its main support from the observed fact that in *Dahlia*, *Lathyrus*

rus, and other forms there is an inverse quantitative relation between anthoxanthin and anthocyanin pigment types. If one is increased through gene substitution, the other is observed to decrease. Apparently a similar competitive relation holds for pigments of the anthocyanin and chalcone types (170). These relations seem most simply explained on the assumption that a common precursor, limited in amount, serves in the formation of all three types of pigment (172, 248). It will be recalled that analogous relations are found in the coat colors of the guinea pig and in eye-color pigments in *Drosophila*.

While there remains much to be learned about flower pigment biosynthesis and its relation to gene systems, it is nevertheless clear from the gratifying progress that has already been made that in many instances genes act in very specific ways. The assumption that each gene functions in a primary way in the control of one specific chemical reaction is certainly strongly supported by work in this field.

I. Disease resistance

Many non-infectious diseases are known to result from deviations in genetic constitution. In a number of these something can be said as to the nature of the correlated metabolic disorders. Several of these concerned with tyrosine metabolism in man have already been mentioned, as has a specific endocrine deficiency responsible for dwarfness in the mouse.

Amaurotic idiocy (Tay-Sach's disease), in some cases at least, appears to be differentiated from normal by a single recessive gene confined largely to members of the Jewish race (326). Clinically it is recognized by gradual development of blindness, idiocy, and paralysis. Metabolically it is characterized by excessive deposition of lipids in the ganglia and glia cells of the brain, spinal cord, and retina (27). Evidently some specific defect in lipid metabolism is responsible.

Defects in porphyrin metabolism are known in man. In porphorinuria, a rare recessive trait, the urine is characteristically red due to porphorin pigments. Porphyrins are present in exposed tissues where they result in photosensitization. Exposure to light results in bulbous eruptions which usually lead to severe scarring and disfigurement (55, 101). A more serious difficulty of the same general nature is found in xeroderma pigmentosum, although the nature of the sensitizing substance is not definitely known. In this hereditary error of metabolism, epitheliomata or, less often, round or spindle-celled sarcomata develop at the site of skin lesions. The disease is semilethal; some two-thirds of those affected die before the age of fifteen years, while only occasionally a sufferer lives to reproduce (55). Xeroderma pigmentosum is incompletely recessive, as shown by the fact that heterozygotes usually exhibit a type of heavy freckling which, unlike certain other types, is not associated with red hair.

Cystinuria, the excretion of 0.4 to 1.0 g. of urinary cystine per day, pentosuria, the excretion of abnormal quantities of the pentose sugar *l*-xyloketose in the urine, and steatorrhea, a defect in fat metabolism leading to the production of "butter stools" and ascribable to a defective functioning of the pancreas, are

all examples of diseases in man in which the difficulty can be ascribed to a particular phase of metabolism. In some cases, each appears to be inherited as a simple recessive trait (27, 101). Although the evidence cannot be said to be unambiguous, diabetes mellitus appears to be markedly influenced by genetic constitution. One of the factors that make this disease difficult to study from the standpoint of genetics is the fact that it often appears relatively late in life, and the classification of individuals with certainty is therefore difficult. The relation of diabetes mellitus to sugar metabolism and insulin is so well known as to require no comment here. Glycogen storage disease, characterized by excessive storage of glycogen in enlarged liver and kidneys, is probably a recessive trait (27).

Even a casual perusal of Cockayne's *Inherited Abnormalities of the Skin and its Appendages* (55), in which over one hundred inherited diseases are listed, Waardenburg's monograph on inherited eye abnormalities (326), Bodansky and Bodansky's *Biochemistry of Disease* (27), and other such works cannot but impress one with the multitudinous ills which defective genes can inspire. The opportunities for advances in our knowledge of human metabolism through studies of these from a biochemical standpoint are almost unlimited. The concept of the genic control of specific biochemical reactions should do much to bolster confidence that such studies can contribute significantly to our understanding of these various errors of function.

Diseases due to nutritional deficiencies at first sight would seem to have little to do with genes. From the standpoint of immediate relation this is often so, but when it is appreciated that nutritional needs are based ultimately on genetic constitution, a more significant relation becomes evident. We are subject to scurvy while the rat is not, because we differ in genetic constitution. The genes in us in immediate control of ascorbic acid synthesis are inactive, while those of the rat function properly. This thesis will be developed more fully in another connection (page 58); it is sufficient here to say that from a genetic standpoint it is possible to have two individuals of a species differing in a single gene one of which requires a given compound in the diet while the other is able to synthesize it from simple dietary components.

In infectious diseases both the genetic constitution of the host and that of the pathogen are significant in determining susceptibility or resistance. This is true for diseases caused by viruses, bacteria, fungi, and protozoa. It is probably also true for diseases caused by metazoan parasites.

In the tobacco mosaic virus disease Holmes (133) has transferred the resistance of the species *Nicotinia glutinosa* to its cultivated relative *N. tabacum*. The transferred resistance is clearly hereditary, although in this particular case it may not be differentiated from susceptibility by one gene only. In a similar way the virus is itself subject to genetic change. Ordinary strains of virus readily infect normal strains of tobacco. But a mutant form known as Jensen's No. 14 strain produces only local lesions (162). The fact that it is a mutant strain is most clearly shown by the fact that it occasionally back-mutates to a virulent strain. Whether we call the virus mutation a "gene" mutation or not

depends on how we regard the virus. In any case the change appears to be analogous to a gene mutation (see page 71). In the case of both resistant host and non-virulent virus strain infections occur but are confined to local lesions that do relatively little harm to the host plant.

An example of genetic differences in susceptibility of a host to a pathogenic bacterium is found in bacterial wilt of Indian corn caused by *Phytophthora stewartii* (331). Resistant and susceptible strains of corn exist and in some cases are genetically differentiated, resistance being dominant. The bacterium is likewise subject to variation. It undergoes mutation to non-pathogenic forms (177), although, since bacteria reproduce only asexually, there is no way of determining whether or not genes are concerned in such modifications. Mutational changes in pneumococci are known to occur from "smooth" (capsulated) virulent to "rough" (non-capsulated) avirulent types and the reverse. Under certain conditions the change from rough to smooth can be accompanied by a change in antigenic specificity and this can be experimentally controlled (6; see page 75). Other bacteria are subject to similar changes in cultural type and virulence (239).

Fungal diseases show similar relations. Rust of maize attacks certain strains but not others. The difference can in certain instances be attributed to a single gene change (197, 244). Analogous differences exist in the susceptibility of onion strains to onion smudge disease (*Collitotrichum circinans*), but here a good deal more can be said about the chemistry of resistance. White strains of onions are subject to the disease but red or yellow ones are not, even when the bulbs are artificially inoculated with the spores of the fungus. The scales of the colored onions contain the polyphenols catechol (1,2-dihydroxybenzene) and protocatechuic acid (3,4-dihydroxybenzoic acid), while those of the white bulbs do not (180, 329). Both protocatechuic acid and catechol are capable of inhibiting the germination of spores of the fungus in solutions at a dilution of about 1:1000, and they are therefore believed to be responsible for the resistance of the colored onions. It is of interest that both of these compounds are related to quecetin, the main pigment of the yellow onion. Red onions contain related pigments not yet completely identified (180). Whether the polyphenols mentioned are precursors of the pigments or breakdown products is not known.

Genetically it is known that pigmented onions may be differentiated from white ones by a single gene pair, pigmented forms being dominant. Yellow differs from red in a separate gene and red is dominant. An additional partially dominant pigment inhibitor gene is known. In the heterozygous form it results in bulbs with pigmented tops (51, 245). It is clear that these genes having to do with pigment synthesis are also concerned with the presence of catechol and protocatechuic acid. If these are precursors of the scale pigments, both the recessive and the dominant genes for white must block the synthesis prior to their formation.

The apple scab fungus *Venturia inaequalis* shows a variation in its pathogenicity toward specific apple varieties that is clearly genetic in nature (146). This is strikingly demonstrated in this sac fungus by the fact that the eight sexual

spores of a spore sac from certain crosses of virulent by non-virulent invariably give rise to four spores like each parent.

Rust and smut fungi are responsible for many diseases of economically important crop plants. They have been shown to exist in many biotypes (genetically homogeneous strains) with characteristic differences in pathogenicity when tested on specific host strains (48). Thus stem rust of wheat has many such biotypes. These undergo hybridization, both in nature and in the laboratory, and as a result gene recombinations giving new biotypes are formed (59, 250). Furthermore, mutation is known to give rise to new strains in this and other rust and smut fungi (47). The plant breeder is constantly attempting, through hybridization and selection, to obtain resistant host plants with otherwise desirable features. We have here an interesting conflict of interest—the wheat breeder endeavors to develop wheat varieties resistant to the existing biotypes of wheat rust, but as soon as this is done, survival of the parasite demands that nature produce through hybridization or mutation new strains of rust capable of surviving on the newly introduced varieties.

A knowledge of the chemical difference between virulence and avirulence as well as that between host susceptibility and resistance would help us to determine what parts are being played by the genes concerned, even though it might not immediately tell us how to establish permanently immune varieties of wheat.

J. Sex phenomena in unicellular organisms

Without doubt the most remarkable series of studies in biochemical genetics is that of the German investigators Moewus, Kuhn, and coworkers on the flagellate *Chlamydomonas*. This work has been reviewed relatively recently by Sonneborn (277) and by Moewus (214). Because of the war, reports are not available for the three years just past.

Chlamydomonas is a unicellular, uninucleate, green, ellipsoidal, biflagellated organism from 5 to 20 microns long. In the free swimming stage the cells are haploid, i.e., the nuclei contain one set of chromosomes. Vegetative reproduction occurs freely under suitable conditions. Some forms are dioecious with gametes of two sex types. These may be morphologically alike or different. Other forms are monoecious with gametes of only one kind. Gametes of different sexes conjugate, if environmental conditions are favorable, giving rise to a diploid zygote. This forms a heavy wall and becomes a zygote cyst. On germination of this, the nucleus undergoes two typical meiotic divisions and four flagellated haploid daughter cells are formed. If the strain is dioecious, two of these are of one sex and two of the other. In monoecious strains any two gamete cells can conjugate; otherwise the life cycle is similar to that in dioecious races.

Chlamydomonas can be cultured in liquid media, either with or without bacteria present, or on agar media. On solid media the cells have no flagellae and of course are not motile. If cells are cultured in liquid in the dark without either sugar or oxygen, they are likewise not motile. In liquid, motility is restored either by light or by a supply of both oxygen and sugar.

types carrying both genes. If the two valencies are equal, these are monoecious lines. Cells with neither *F* nor *M* genes are lethal.) Each form of the gene *F* determines a ratio of *cis*- and *trans*-molecules of the polyene as follows:

<i>F</i> allele	Ratio <i>cis</i> -polyene: <i>trans</i> -polyene
<i>F</i> ⁵	98:2
<i>F</i> ⁴	95:5
<i>F</i> ³	85:15
<i>F</i> ²	75:25
<i>F</i> ¹	65:35

In a similar way the five alleles of the *M* gene determine that the ratio of *cis*- and *trans*-polyene shall be as follows:

<i>M</i> allele	Ratio <i>cis</i> -polyene: <i>trans</i> -polyene
<i>M</i> ¹	35:65
<i>M</i> ²	25:75
<i>M</i> ³	15:85
<i>M</i> ⁴	5:95
<i>M</i> ⁵	2:98

The assumed polyene precursor is converted to protocrocin as indicated in the scheme. The gentiobioside shown is formed in *C. engametos*, but *C. dresdensis* forms the analogous cellobioside, while in *C. braunii* this is replaced by the cellotrioside. The specific sugar present is determined by which of three alleles of the *flac* gene is present. The ratio of *cis*- to *trans*-isomers determined by the *F* or *M* allele present is maintained during protocrocin formation.

Under the control of the gene *mot*, presumably through the mediation of a specific enzyme, protocrocin is cleaved to crocin and picrocrocin or a related compound. Again the ratio of *cis*- to *trans*-forms of crocin is maintained. Crocin is the motility hormone. Its activity is astoundingly high, 1.2 molecules per cell being sufficient to cause two flagellae per cell to develop within a period of 20 min. Both isomers are active.

The terminal group glycoside (possibly gentiobioside) formed on the cleavage of crocin functions as the so-called gynotermone. It is capable of determining that the cells of a monoecious culture become females.

Under the influence of two completely linked but not allelic genes, *gathe_{cis}* and *gathe_{trans}*, two enzymes are produced which oxidize the two isomers of crocin to the corresponding *cis*- and *trans*-isomers of crocetin dimethyl ester. The enzyme which converts *cis*-crocetin is active either in light or darkness, but the *trans*-enzyme is active only in the light. In the light, therefore, *cis*- and *trans*-crocetin dimethyl esters are formed in the original ratio determined by the *F* and *M* genes. The mixtures of *cis*- and *trans*-crocetin dimethyl esters in the ten ratios indicated above constitute the ten gamones. Each renders cells of corresponding valence capable of conjugation. In the dark only *cis*-crocetin dimethyl ester is formed and this by itself has no gamone activity. It can be converted to the *trans*-isomer through a photochemical reaction, as Kuhn and Winterstein have shown.

In male cells or cells of monoecious strains which carry the gene M_D closely linked to M , an enzyme is formed which is capable of splitting the sugar from gynotermone to give "androtermone" (*l*-4-hydroxy-2,6,6-trimethyl- Δ^1 -tetrahydrobenzaldehyde). This substance is capable of determining that cells of monoecious races shall be male in reaction (161). In females the M_D gene is not present, and therefore no androtermone is produced by them.

Aside from the F and M and $flac$ genes, in each of which several alleles occur naturally, the genes indicated in figure 5 were detected by Moewus by inducing mutations in them. Thus, if the *mot* gene is inactivated, both motility hormone and gynotermone fail to appear. That production of both substances is under the immediate control of a single gene is shown by the fact that in all of sixty-four recurrences of the same single-gene mutation, production of *both* hormones was blocked. If the M_D gene is inactivated in cells that would otherwise be males, there result individuals with female termone but male gamone. The *gathe_{cis}* and *gathe_{trans}* genes are capable of being independently inactivated through mutation. Strains that cannot convert either *cis*- or *trans*-crocin can be obtained by first inducing a mutation in one *gathe* gene and subsequently causing the remaining one to undergo change.

As regards manner of action, *Chlamydamonas* genes *mot*, M_D , *gathe_{cis}*, and *gathe_{trans}* appear to control specific enzymes which in turn catalyze specific reactions. The *gathe_{trans}*-controlled enzyme has the unusual property of acting only in light. The *flac* alleles are assumed to work by way of enzymes specific to the type of sugar residue of procrocin. If *flac^{gent}* is present, the sugar is gentiobiose; if the *flac^{celb}* allele is substituted the cellobioside is synthesized; while the *flac^{celtri}* allele determines that the sugar will be cellotriose. The F and M series of alleles have still more remarkable properties. It will be recalled that they determine in what proportions the *cis*- and *trans*-isomers of the polyene precursor will be synthesized. How this is accomplished is not known with certainty, but it is proposed by Kuhn and Moewus that the mechanism is possibly similar to the one determining that amino acid residues in proteins will occur in ratios according to the Bergmann-Niemann numbers. While this is by no means a completely satisfying interpretation, it is remarkable that the *cis-trans* ratios do fall into a series that can be approximated by Bergmann-Niemann numbers. The agreement is indicated in the following comparison for female sexes (277):

SEX	VALENCE	BERGMANN-NIEMANN NUMBERS	Cis-trans RATIO OF DIMETHYL ESTER OF CROCETIN	
			Calculated	Observed to be active
Female	1	2:1	66.7:33.3	67-64:33-36
	2	3:1	75.0:25.0	75-74:25-26
	3	2 \times 3:1	85.7:14.3	86-83:14-17
	4	2 \times 3 ² :1	94.7: 5.3	96-94: 4- 6
	5	2 \times 3 ³ :1	98.2: 1.8	99-97: 1- 3

A similar agreement is found for the five male types with the ratios transposed.

It is most unfortunate that after presenting so beautiful an interpretation

and one which agrees with so many of the reported facts, one must introduce a note of skepticism. The facts reported and the interpretation are almost "too good to be true." As a matter of fact, Philip and Haldane (232) have, on the basis of a statistical analysis of certain of Moewus' genetic results, made just such a criticism. Accepting the interpretation at face value and then examining the distribution of sampling errors, they come to the conclusion that, "... if every member of the human race conducted a set of experiments of this type daily, they might reasonably hope for such a success once in 50,000 million years." Moewus (214) has attempted to reply to this most serious criticism. Patau (229) has analyzed other data of Moewus with a similar conclusion (see also Ludwig (189)). Sonneborn (277) has subjected the Kuhn-Moewus work to a detailed and searching criticism and concludes that it is most important that the work be repeated by investigators working independently. The writer is in complete agreement with this sentiment.

A second case of sex differentiation in a unicellular organism of particular interest to biochemical geneticists is that found in the ciliate protozoan *Euplotes patella*. Kimball (150, 151) has observed that in this organism, in which the individuals are normally diploid, there are six mating types. Genetic studies show that these types are determined by three allelic forms of one gene. The three homozygotes mt^1mt^1 , mt^2mt^2 , and mt^3mt^3 represent three types, while the three heterozygotes, mt^1mt^2 , mt^1mt^3 , and mt^2mt^3 , give rise to the remaining three types of mating behavior. Biochemically it is found that culture filtrates contain specific substances which are responsible for conjugation reactions. There are three such substances, each controlled by one of the mating type alleles. The three homozygous types therefore each produce one of these sex hormones, while each of the three heterozygotes produces one of the three possible combinations of two substances. An animal may be activated by any substance other than the one or two specific to itself. Unfortunately, the chemical nature of these substances has not yet been determined. The gene-reaction correspondence is here very striking, and in the apparent independent action of alleles in controlling the production of different specific substances we have a resemblance to the gene-antigen relation to be discussed below.

The ciliate genus *Paramecium* is similar to *Euplotes* in that several infertile mating types may be present within one species, but it apparently differs in not releasing mating-type substances into the culture medium. There is, however, evidence that specific substances which may be transferred from animal to animal by contact are concerned (278). For further details regarding mating types in species of *Paramecium* the reader is referred to recent papers by Sonneborn (278) and by Jennings and Opitz (142).

K. Genes and immunological specificity

Antigens are known to be substances of high molecular weight (10,000+). They are often proteins, though apparently polysaccharides may exhibit antigenic properties (30, 165). Antigenic specificity may be determined by relatively simple compounds, the so-called haptens of Landsteiner, combined with proteins. An antigen of one species injected into the blood of another under

the proper conditions may induce the formation of an antibody specific to the inducing antigen. Antibodies are often, if not always, serum globulins. The antigen-antibody reaction is the result of an intimate union between the two, probably involving hydrogen bonds and at least under certain circumstances reversible. This union may result in any one of several reactions, such as agglutination, precipitation, lysis, or toxin neutralization. The antigen-antibody reaction may also be detected by complement fixation (reviews: 30, 165).

The antigens characteristic of a species are intimately related to its genetic makeup. This is well illustrated in the blood groups of man. The first of these, the A-B blood groups, were first discovered in 1900 by Landsteiner (164). It has subsequently been determined that the four main groups, O, A, B, and AB, are genetically determined by the three alleles I^O , I^A , and I^B (reviews: 301, 335). Of these I^O is inactive, I^A is correlated with antigen A, and I^B conditions the presence of antigen B. Both I^A and I^B are dominant to I^O , but when I^A and I^B are present in the same individual, neither is dominant, but determine that both antigens A and B will be present. The antigens A and B are found in red blood cells and in other tissues; they are known as hemagglutinogens. Antibodies, known as hemagglutinins, are normally present in sera if their corresponding antigens are absent. Summarizing these genetic and immunological relations, we have the following:

BLOOD GROUP	GENETIC CONSTITUTION	ANTIGENS IN CELLS	ANTIBODIES IN SERUM
O.....	$I^O I^O$	None	α, β
A.....	$I^A I^A$ or $I^A I^O$	A	β
B.....	$I^B I^B$ or $I^B I^O$	B	α
AB.....	$I^A I^B$	AB	None

The agglutinins α and β correspond to the agglutinogens A and B. If cells and sera with either one or two pairs of corresponding agglutinogens and agglutinins are mixed, agglutination of cells occurs. A subgroup of type A is known, and the antigen characteristic of it is dependent on a fourth allele of the series (335). This, however, does not alter the principles involved in either the inheritance or the serological action.

The M-N blood types, discovered in 1927 by Landsteiner and Levine (166), differ from the A-B groups in that antibodies are not normally present but must be produced through immunization. For this reason the M-N blood types are not clinically important in blood transfusion. The M-N types are determined by the two alleles A^M and A^N , which are independent in inheritance of the A-B alleles. There are three genotypes possible and they correspond to the three blood types as follows:

GENOTYPE	BLOOD TYPE
$A^M A^M$	M
$A^M A^N$	MN
$A^N A^N$	N

Again each allele is related to a specific antigen, and if both are present they act independently, each producing its characteristic antigen. There is no inactive allele of this gene known.

In connection with their early work on the M-N blood types Landsteiner and Levine discovered a hemagglutinin in man immunologically different from those of the A-B or M-N groups. This has become known as agglutinin P. Antibodies against it may occasionally occur normally in human sera but usually are not present. The presence of P is inherited as a simple dominant trait. About 24 per cent of the individuals classified proved not to carry the P agglutinin, i.e., were homozygous recessive for the gene concerned with its production. The available information on the occurrence of the P antigen, its properties, and its inheritance have been summarized by Wiener (335).

A fourth series of blood types in man, first reported by Landsteiner and Wiener in 1940 (168), is designated the Rh series after the Rhesus monkey in which the Rh antigen was first found. According to Wiener (336) there are six alleles of a gene independent of the A-B and M-N genes responsible for the Rh antigenic specificities. One of these, *rh*, is inactive. Three, *Rh₀*, *Rh'*, and *Rh''*, directly control the production of three corresponding antigens (see also Murray (220)). The remaining two, *Rh₁*(*Rh'₀*) and *Rh₂*(*Rh''₀*), each produce antigens with the combined specificities *Rh₀* + *Rh'* and *Rh₀* + *Rh''*. These various alleles determine antigens detected with special antisera. As tested with standard Rh antiserum, about 15 per cent of the individuals of this country are Rhesus-negative (genetically *rh rh*), while the remaining 85 per cent are Rhesus-positive (*Rh Rh* or *Rh rh*). Again, as in the M-N types, antibodies are not normally present in the serum of Rh-negatives but can be produced through immunization either in man or in other animals. An interesting relation arises when an Rh-negative mother carries an Rh-positive fetus. The Rh antigens of the fetus may, under circumstances not yet clearly understood, leak through the placenta and induce antibody formation in the mother's blood. These antibodies may then pass back through the placenta and lyse the red blood cells of the fetus in the same or a subsequent pregnancy. This situation may lead to prenatal death, to a serious postnatal state of the infant known as erythroblastosis fetalis, or possibly, as recently indicated, to permanent mental impairment (276a). It is only under the specific genetic relations indicated—an Rh-negative mother, an Rh-positive fetus, and of course an Rh-positive father—that erythroblastosis fetalis results. Fortunately, the difficulty arises in only a small fraction of the pregnancies. For further details and references the reader is referred to Wiener's excellent summary of the Rh and other blood antigens of man (335).

A most illuminating study of genetically controlled interspecific variation is that of Irwin, Cole, and Cumley on the pigeons and doves (family *Columbidae*). Hybrids involving the two species *Columba guinea* (wild pigeon) and *C. livia* (domestic pigeon) and three species of dove, *Streptopelia chinensis* (Pearlneck), *St. risoria* (Ring dove), and *St. senegalensis* (Senegal) have been made in various combinations, and they and their progeny in back-crosses to pure species have been studied with respect to blood-cell agglutinogens and serum antigens

(for reviews and references see 61, 139, 140, 141). In principle these findings can be summarized as follows: For any two species there are both common and species-specific cellular antigens which can be detected with appropriate antisera. Hybrids between the two species have the antigens common to the two plus those specific to both parents. Thus, in the Pearlneck-Ring dove hybrid there may be present cellular antigens A B C D, whereas Pearlneck has only A B C and Ring dove only B C D. A is specific to Pearlneck and D to Ring dove, while B and C are common to both. Back-crosses of the F_1 hybrid to the parental species show that species-specific antigens are inherited as though they were determined by dominant genes. The Pearlneck-Ring dove hybrid back-crossed to Ring dove shows that there are at least ten cellular antigens specific to Pearlneck. Since the genes determining these are heterozygous in the F_1 , while the Ring dove parent is homozygous recessive, the back-cross progeny segregate in a one-to-one ratio of presence and absence of each of the ten Pearlneck-specific antigens. The analogous back-cross of the hybrid to Pearlneck is very difficult to make, and accordingly relatively few offspring have been tested. Such tests as have been possible, however, indicate that at least nine cellular antigens are specific to the Ring dove parent (139).

If the cellular antigens of the two species, Pearlneck and Ring dove, are compared with still other species, e.g., *C. livia* and *C. guinea*, it is found that certain of the antigens shared by Pearlneck and Ring dove are specific to *C. guinea* as compared with *C. livia*. Other interspecific comparisons show similar relations.

The study of antigenic differences in *Columbidea* has recently been extended to serum antigens (60, 62). In successive back-crosses of Senegal-Ring dove hybrids to the Ring dove parent, at least three distinct serum antigens have been identified and shown to be inherited in a mendelian fashion. The genes responsible for these appear to assort independently of those determining the several (possibly ten or more) Senegal cellular antigens not present in Ring dove.

Individual chickens are known from the work of Landsteiner and Levine (167), Todd (320, 321), Thomsen (318), and others to vary in their blood-cell antigens. This variation is known to be inherited in a manner essentially similar to that of the blood groups of man. Wiener (335) has pointed out that certain of the data of Todd can be interpreted on the basis of three alleles of one gene, each producing a specific agglutinin, plus an independent gene pair with a dominant allele controlling the production of a fourth agglutinin and an inactive recessive allele. Ducks have been investigated by McGibbon (195) and others. The Mallard and Muscovy show relations similar to those found in the pigeon-dove group. Each contains species-specific cellular antigens as well as a group of antigens common to both. Since the hybrid between these species is sterile, direct tests of the hereditary basis of species-specific antigens is not possible. However, genes determining them must be dominant, since the antigens are present in the F_1 generation. Intraspecific antigenic differences are known to be inherited in these species, the presence of an antigen being dominant to its absence.

Wiener (335) has summarized available data on individual variation in cellular antigens in various mammals. Apes and monkeys show blood group variation much like that of man; in fact, homologous agglutinogens can be identified in many instances. There are few or no inheritance data available in these animals. Heritable cellular antigens are known in cattle, sheep, rabbits, mice, rats, and dogs. In each case the presence of a specific antigen parallels the dominant allele of a specific gene. An interesting expression of immunological differences is found in animal tissue transplants. It has long been known that tissue—for example, skin—can be successfully transplanted from one location to another in a single individual, but not usually from one individual to another. Loeb and Wright (186) found this to be so in guinea pigs. But if lines of guinea pigs, inbred by brother-sister matings for a sufficient number of generations to insure that substantially all genes become homozygous, are used, transplants between individuals are successful. If two inbred lines, each of which fails to accept grafts from the other, are crossed, the F_1 hybrid will accept grafts from either parent. The parent lines, on the other hand, will not accept grafts from the F_1 hybrid. These relations are interpreted genetically on the assumption that certain genes are concerned with the success of the transplant and that they act in such a way that an animal will not accept a graft containing dominant alleles which it does not itself have. On the other hand, such dominant alleles in the host are without effect regardless of whether the graft has them or not. Immunologically it has been supposed (127, 307, 349) that these dominant genes are concerned with antigen formation. If a graft contains antigens not shared by the host, antibodies against the transplanted tissue are induced and they interact with the graft antigens in such a way as to cause the graft to be cast off or resorbed. Schematically this situation can be represented as follows:

	GENETIC CONSTITUTION	ANTIGENS IN TISSUES
Line 1.	$AA\ bb$	A
Line 2.	$aa\ BB$	B
Hybrid.	$Aa\ Bb$	A, B

It is readily seen that of the six possible host-donor combinations, in only two (Line 1 on hybrid and Line 2 on hybrid) will the host have all the antigens of the graft. The results in the F_2 generation and in back-crosses will depend on how many antigenic differences exist between the two lines and how they are distributed. By the methods of genetics these relations can be determined. Similar relations appear to hold for tissue transplants in mice (182). In rats the situation appears to be somewhat more complex and has not yet been analyzed on a basis as simple as that just given (185). As will be pointed out later (page 53), essentially similar relations often hold when tumor tissues are transplanted from one individual to another in experimental animals.

In all instances so far presented it appears that a one-gene-one-antigen relation obtains. Actually, while this is the relation in the great majority of cases that have been studied, there are several notable exceptions. In the dove

hybrids—for example, Pearlneck by Ring dove—there appear so-called “hybrid substances,” i.e., cellular antigens not present in either parental species (140). Analogous hybrid antigens are found in the Mallard-Muscovy duck hybrid referred to above. Occasionally antigens of an apparently similar nature appear in chicken crosses (318). In the dove and duck hybrids these appear to be the result of two or more complementary dominant genes, one or more contributed by each parent and possibly having to do with species-specific antigens. Thomsen suggests that in the chicken they may represent recessive antigenic determiners that were masked in the parents, but the complementary dominant gene theory would appear to fit the facts equally well. The usual one-to-one gene-antigen relation suggests, as has often been pointed out (129, 130, 140, 347), that antigens may be direct gene products. This, of course, is in agreement with the general working hypothesis of gene action presented earlier in this paper. The antigen, or at least the part of it responsible for its specificity, may well be a direct copy of a gene. The antigens dependent for their production on two or more complementary genes would, on this hypothesis, have their specificities determined by two components, each copied from a different gene.

An interesting biological application of immunological specificities is found in “serological systematics.” It is found that the degree of antigen difference between any two species is correlated with their systematic divergence as determined on morphological grounds. On the assumption that antigens in general are gene determined, measurements of serological relationships measure degrees of relationship in terms of a particular category of genes. By and large, these would be expected to give a fair picture of relationships in more general terms. Extensive studies in both plant and animal kingdoms have been made on this basis (reviews: 31, 45) and have contributed information of substantial value to the systematist.

L. Self-sterility in plants

Evidently in most plants and animals there is a strong selection against close inbreeding, for there exist several well-developed mechanisms for preventing its occurrence. One of the most interesting of these from the viewpoint of gene action is that responsible for self-sterility or self-incompatibility. In the early days of plant genetics it was observed that many species of monoecious plants cannot be successfully self-fertilized but can be crossed freely with other plants even though these be relatively closely related. The phenomenon is widespread in the plant kingdom (81). The most prevalent genetic basis of it is the oppositional allele mechanism first worked out for tobacco by East and Mangelsdorf (82). In this and many other plants there exist many forms or alleles of a specific gene, and these act in such a way that a pollen tube (haploid generation) carrying one of these alleles will not grow sufficiently fast to bring about fertilization in a style (diploid maternal tissue) that carries this same allele. Thus an S_1S_2 plant produces both S_1 and S_2 pollen, but neither type will grow in the S_1S_2 style, although both will function perfectly in an S_3S_4 style. In a mating such as $S_1S_2 \times S_1S_3$, half the pollen (S_3) functions. Some fifteen such alleles were

identified in tobacco. In the evening primrose *Oenothera organensis* Emerson (84) has shown that there are at least forty-five such alleles of a single gene. In the clover, *Trifolium repens*, Atwood (3) has found as many as thirty-nine alleles in a relatively small population. Actually there must be many more than this. With random distribution of such a large number of alleles in a population the chance of any two plants being identical in constitution and hence cross-sterile is of course small. Wright (345) has given a mathematical analysis of the distribution, mutation frequencies, etc. of self-sterility alleles in *Oenothera organensis*.

Although it has often been suggested that the incompatibility reaction between style and stigma is similar to an immunological reaction, only a beginning has been made in interpreting self-sterility in physiological and immunological terms (84, 175a). From the standpoint of structure and function of the gene, the remarkable aspect of the self-sterility alleles is that there are so many forms possible of a single gene, each maintaining its autocatalytic property and at the same time imparting a unique specificity to the pollen tubes and tissues of the style. In clover, judging from Atwood's results, one might guess that there are at least a hundred different alleles possible.

M. Genes and cancer

The problem of the nature and causes of cancer is a tremendous one and one on which a voluminous literature has grown up. There is as yet no general solution, but it can nevertheless be said that genes are of basic importance. In one sense, abnormal cell proliferation may be regarded as a defect in normal differentiation. Instead of acquiring a definitive form and becoming a normally integrated part of a specific tissue, cancerous cells retain a high division rate. In this respect they are like embryonic cells, but in other regards they differ, e.g., in usually having a granular cytoplasm, in the appearance of cytoplasmic constituents, and often in irregularities in chromosome behavior.

For many years it has been known that for many transplantable tumors the ability of the tumor cells to survive in the host is dependent on both the genetic constitution of the tumor cells and that of the host into which they are put. Little and Tyzzer (184), Little and Strong (183), Cloudman (54), Bittner (24), and many others have found that the behavior of a mouse tumor graft is subject to the same principle as is a normal tissue graft (page 51). If a tumor arises in a pure line, it can be transplanted to other individuals of the same line but not usually to strains of other genetic constitutions. If two inbred lines, one susceptible to a tumor transplant and the other resistant, are crossed, the F_1 mice are susceptible. In back-crosses to the resistant line a segregation of resistant and susceptible mice is found, the numerical relations of which indicate the number of genes concerning tumor resistance by which the parent strains differ. As in normal tissue transplants, the requirements for a successful transplant are that the tumor tissue have no dominant alleles of pertinent genes which are not also present in dominant form in the recipient. Sometimes the significant

difference may be in only a single gene in which case the situation will be as follows:

Parents (1)	<i>aa</i> (resistant)
(2)	<i>AA</i> (susceptible)
Tumor (spontaneous in (2))	<i>AA</i>
 F ₁ hybrid	 <i>Aa</i> (susceptible)
 Back-cross F ₁ to (1)	 1 <i>Aa</i> (susceptible) 1 <i>aa</i> (resistant)
 F ₂ generation	 1 <i>AA</i> (susceptible) 2 <i>Aa</i> 1 <i>aa</i> (resistant)

Usually strains differ by a larger number of significant genes, often as many as six or eight. If transplants of tumors arising in the F₁ hybrids between strains are made, the F₁ is susceptible but the two parental strains are resistant. The back-cross and F₂ generation segregations indicate the number of genes concerned and agree with one another (303). An immunological interpretation can be applied to these results just as in the case of normal tissue transplants (127). If the tumor carries one or more dominant genes conditioning the presence of corresponding antigens and the host has inactive or immunologically different alleles of these genes, the tumor graft induces antibody formation against itself and it then retrogresses. Several types of transplantable tumors in mice have been shown to behave in this manner (reviews: 26, 181, 275). MacDowell and associates (246), as well as others, have shown that a similar genetic interpretation can be made of differences in susceptibility to transplantable leukemia (cancerous leucocyte-like cells that circulate in the blood stream).

It has been observed that occasionally transplantable tumors in mice undergo spontaneous changes in growth properties. A number of these have been shown by Strong (302), Cloudman (53), and Bittner (24) and others to involve genetic simplification, in that the modified tumors require that fewer dominant genes be present in the host for successful growth than were required by the original tumor. Mutations of this type, which may of course involve physical loss of chromosome segments containing the relevant genes, may have an important bearing on the origin of transplantable tumors which are not strain-specific. If a tumor, originally containing gene-controlled antigens that render it strain-specific, is put in a host capable of building antibodies against it, then such a host will act as an enrichment culture for any mutant tumor cells which have lost antigen-determining genes either through true gene mutation or through physical loss. Loss of all species-specific antigen-genes would of course result in a tumor without any strain specificity. An alternative hypothesis involving masking of antigens through mutant changes has been proposed by Gorer (114).

That there is an immunological basis of tumor specificity has been experimentally demonstrated by Gorer (113), who showed that a sarcoma originating in one strain of mice actually induced agglutinin formation in another strain in

which tumor transplants showed regression. The two strains differ by two or three significant genes, and one of these was shown to be identical with one shown in independent experiments to control the production of hemagglutinin II. A similar situation appears to obtain with regard to genes for susceptibility to transplantable "stem-cell" leukemia in mice; one of the genes concerned is reported to be identical with the hemagglutinin II gene (115). Antibodies specifically directed against the Brown-Pierce tumor have been reported in rabbits (147). Reference is made to Bittner (25, 26), Snell (275), and Little and Gorer (181) for further details and reference to the literature on transplantable tumors.

A number of specific compounds are known to increase the incidence of tumor origin. Notable for effectiveness among these carcinogenic substances are 1,2,5,6-dibenzanthracene, 3,4-benzopyrene, 20-methylcholanthrene, 9,10-dimethyl-1,2-benzanthracene (reviews: 36, 181, 275). So far as genetic aspects of tumor induction go, two main questions arise, *viz.*: (1) does genetic constitution influence the response of the organism to carcinogens, and (2) are carcinogens effective through inducing mutations? A discussion of the second question is deferred (see page 57). To the first the answer is yes. It is known that strains of mice differ in their responses to carcinogenic agents (reviews: 181, 275), but the detailed genetic basis of this difference is not known.

Tumors arising without treatment are known in many animals, both invertebrate and vertebrate. Because of the obvious relation to cancer in man, mammals such as the mouse have been most studied with regard to these.

In the vinegar fly *Drosophila melanogaster* the development of spontaneous benign larval tumors is known to be genetically conditioned (253). In addition, a recessive sex-linked lethal is known in which all males carrying the mutant allele develop melanotic lesions at the femur-tibia junction (117).

Kosswig and Gordon have found that hybrids of the two genera of viviparous top minnows, *Platypoecilus maculatus* and *Xiphophorus hellerii* (Mexican swordtail), having a specific genetic constitution develop melanotic overgrowths (111, 112, 157, 158). The requirement for the development of such melanomas is that the hybrids carry the sex-linked dominant gene for macromelanophores from the platyfish parent. Since in the platyfish parent such melanophores do not undergo excessive proliferation, it is clear that the contribution of the swordtail to the hybrid is important in such unregulated growth. Breeding studies make it highly probable that this contribution consists of partially dominant genes. We have here a clear case of genetic regulation of the growth of a particular type of cell.

Marked variations in the incidence of various types of tumors are known in different inbred strains of mice (reviews: 26, 181, 275). For example, lines are known in which approximately 90 per cent of the females develop breast cancer, while in others the incidence is below 1 per cent. Since the observed incidence varies with age, the values for particular lines are subject to strong modification by environmental factors, e.g., nutritional variations that influence the length of life of a mouse. The difference between high- and low-tumor lines is

clearly subject to genetic control, although the exact genetic basis cannot be determined. It has recently been shown that, in addition to genetic factors, there is a milk-borne agent concerned. Bittner and others have established that this has many of the properties of a virus (9). For the development of this particular type of tumor it is apparently necessary that the milk-borne factor be present and that the genetic constitution of the host be favorable to its multiplication and expression.

MacDowell and coworkers, as well as others (87, 193, 246), have shown that the occurrence of spontaneous leukemia in mice is strongly influenced by genetic constitution. Again, high-incidence and low-incidence inbred lines are known. They evidently differ by at least several significant genes, judging from the results of crosses between them. In addition, there is evidence of extrachromosomal factors, though the nature of these has not yet been established (181).

Similar relations no doubt hold in the occurrence of tumors in man. The hereditary disease xeroderma pigmentosum has already been mentioned. Here, as Haldane (127) points out, it is clear that while exposure to light is evidently the immediate cause of tumor origin, genetic modification is responsible for the abnormal photosensitivity. This relation illustrates the obvious general principle that both genetic constitution and environment are indispensable components of the living system and that it is senseless to argue in any case that one is more or less important than the other. This does not of course deny that a given property of the system may be more susceptible to modification through defined variations in one component than in the other. In the case under discussion there are obvious ways of modifying the end result by varying either a gene or the light exposure.

Neurofibromatosis (von Recklinghausen's disease) is apparently inherited in man as a simple dominant character (55). It is characterized by local pigmentation of the skin, tumors of the skin, and tumors of the peripheral nerves. Most sufferers do not live to reproduce, and consequently the condition is limited to a few generations in a given pedigree. Obviously, to maintain its frequency in the population, it must repeatedly recur through gene mutation. Still other strong predispositions to specific types of tumors in man are known to be inherited (55, 130), and there can be no doubt but that the occurrence of tumors in general has an important genetic component.

From the viewpoint of genetics two questions as to how tumor cells arise are of basic importance. These are: (1) how do such cells differ from normal cells? and (2) how does the characteristic difference arise? It is possible that the difference is genetic, in which case it must arise through somatic mutation. This hypothesis, the origin of cells with unregulated growth characteristics as a result of localized gene mutation, has been proposed many times. The difficulty is that no one has yet devised a method of directly subjecting it to experimental test. This of course cannot be done by the classical methods of genetics, because the somatic cells in which tumors arise leave no descendants by sexual reproduction. There are, however, a number of indirect arguments that bear on it. It is, for example, consistent with the observation that tumors frequently

arise in local regions, probably in single cells. A second argument is of a deductive nature. If genes do control specific steps in metabolism, there must be genes which serve to integrate cell division in different parts of the organism. Many genes, if not all, are subject to change, through either mutation or loss, and unless those having to do with keeping cell division in check are immune to such a change, it must be possible for unregulated growth capacities to arise through mutant changes in them. If somatic mutations are a frequent cause of cancerous properties of cells, then one might expect the carcinogenic agents referred to above to cause genes in general to undergo mutation. While x-rays and ultraviolet radiation are known to induce mutations, this has not been found to be so in the tests that have been made with carcinogenic chemicals. While this does not disprove the hypothesis, it certainly does not argue in favor of it. From numerous investigations on the genetic basis of antigenic differences, such differences are usually if not always to be ascribed to specific gene differences. It would appear at first thought, then, that the demonstration of an antigenic difference between a tumor and the tissue of the animal in which it arose—or others of the same genetic constitution—would strongly indicate that the tumor had arisen through somatic mutation. There are, however, at least two serious objections to this argument in its simplest form. The first is the possibility that the immunological specificity of the tumor may be due to an abnormal cell constituent such as a virus. This may be so even though the virus cannot readily be obtained free of cells. Of course, such a virus might conceivably arise from a normal cell constituent (see page 84), in which case the tumor origin might still be regarded as a special type of somatic mutation. A second weakness of the argument is that there is some evidence for the existence of organ-specific antigens (330). In the case of the rabbit lens antibody of Guyer and Smith (125, 126), for example, it would appear that the lens protein is antigenically different from proteins of other tissues and organs (307). Antibodies are presumably not normally induced against it because it is restricted to the lens. Cellular antigens specifically concerned in the M-N and Rh blood groups appear to be restricted to erythrocytes. It may well be, therefore, that tumors are not antigenically different from the specific cells from which they arise. MacDowell and his associates (193, 194, 246) have shown that mice of an inbred line can be immunized against transplantable leukemia originating in the same line. But, curiously, such immunization does not protect against spontaneous leukemia. This and other aspects of immunity to mouse leukemia are discussed by MacDowell (193). Similar relations are known for other transplantable neoplastic cells (116, 120, 147). It is clear that more information about tissue- and organ-specific antibodies is needed before arguments along the above lines can be pushed further with profit.

If tumor cells differ from normal cells not genetically but in a manner analogous to that by which one type of normal cell differs from another, i.e., in some unknown but non-genic way, genes may still be important in their origin just as genes are important in determining the pathways of normal differentiation. An example of a gene that acts in this way is found in the genetically

recessive polymitotic character in Indian corn (12), in which the four products of sporogenesis fail to undergo the normal growth phase but immediately undergo a series of divisions in which, without multiplication, the ten chromosomes of the original cells are distributed among the daughter cells. This process continues until the supply of chromosomes is exhausted. These unregulated cells are not cancerous because their division potentialities are not unlimited. But they do indicate a principle that could well be concerned in tumor cell differentiation. Furthermore, genetic control of mutation rates may be differentially effective for various tissues (71).

It has been indicated that transplantable tumor cells may undergo spontaneous changes in transplantability, i.e., they may become less specific as to their host requirements. Since this specificity is apparently antigenically and genetically determined, the changes in specificity almost certainly represent gene mutations in the cancer cells. Although there need be no direct relation between mutations of this type and those that result in malignancy in the first place—assuming such mutations to occur—there is a parallel in that in both cases the organism serves as an enrichment culture for those cells with the highest growth rate. Looked at in this way and assuming the somatic mutation theory to be correct, the remarkable thing is not that malignancy changes are as frequent as they are but rather that they are not more frequent.

While the somatic mutation theory of cancer savors somewhat too strongly of fatalism for maximum comfort, it nevertheless cannot be disregarded. And ways of demonstrating its truth or falsity may yet be found.

As is well known, there are a number of tumors in which transmission can be accomplished in experimental animals by cell-free filtrates. These are definitely related to genes insofar as susceptibility to the filterable agent or virus is dependent on genetic constitution. If genes and viruses have as much in common as is thought by some, the relation may be much more direct. In fact, it appears possible that the virus and somatic mutation theories of cancer may not be mutually exclusive in any fundamental way.

N. Biosynthetic processes in Neurospora

If there does exist a one-to-one relation between genes and specific reactions, it should be possible to select from a series of induced gene mutations those concerned with particular reactions. Beadle and Tatum (19) have devised an experimental procedure for doing this with the fungus *Neurospora*. This organism was chosen because it can readily be grown in pure culture on a chemically defined medium, and because its life cycle is conveniently short and otherwise particularly favorable for genetic studies (178, 266).

This organism, commonly known as red bread mold, is heterothallic, that is, exists in two morphologically identical but physiologically different sexes or mating types. Each of these is haploid (seven chromosomes (192)) and by itself reproduces only vegetatively by mycelial growth or through the mediation of asexual spores. The hyphae of the growing mycelium are multinucleate. If mycelia of the two sex types are grown together on a suitable medium, hyphal

fusions occur and fruiting bodies containing sexual spores develop. These spores are produced in sac-like structures known as asci, and their development to maturity at 25°C. requires about 12 days from the time of fusion. The ascospores normally occur in sets of eight, one set per ascus, and they are arranged in a linear fashion like eight eggs in a narrow stocking.

The eight spores of a single ascus arise as a result of three nuclear divisions of an original zygote nucleus, i.e., the diploid nucleus resulting from fusion of haploid nuclei from the two parental strains. The first two of these divisions are meiotic, i.e., reduce the chromosomes from the diploid to the haploid condition. The third division is mitotic and simply divides each of the four meiotic products equationally, giving pairs of spores the members of each of which are genetically identical. The geometrical relations are such that if the parents differ in the alleles of a single gene, the eight ascospores of an ascus are of two kinds, four like one parent and four like the other. Their arrangement may be in two groups of four, one group like each parent, or in alternating pairs of the two parental types, depending on whether or not a crossover occurs between the segregating gene pair and the centromere. The relative frequencies of the two arrangements is a function of the distance of the segregating gene from the centromere. (See Lindgren (178) for further details.)

✓ The nutritional requirements of *Neurospora* consist of (1) a carbon source (any of a number of sugars, starch, fat, etc.), (2) a nitrogen source (NO_3^- , NH_4^+ , or any of several forms of organic nitrogen), (3) inorganic salts providing SO_4^{--} , PO_4^{--} , Ca^{++} , and K^+ , a series of so-called trace elements (136), and (4) the B-group vitamin biotin. Satisfactory growth is obtained either in liquid or on a semi-solid (agar) medium if oxygen is supplied.

✓ The method followed in obtaining biochemical mutants involves treating asexual spores with x-rays or ultraviolet light, making crosses with strains of the opposite sex, and then establishing single ascospore strains. On the basis of the life cycle indicated, such strains should be internally genetically homogeneous. They are grown on a medium as complete as possible in vitamins, amino acids, and other substances, the syntheses of which might be blocked as a result of gene mutations. Thus if such a strain no longer is able to synthesize thiamin, it can obtain this essential material from the medium. Loss of synthetic ability is detected by transferring asexual spores of the individual strains to a medium containing the minimal requirements of the wild-type strain. Failure to grow on this indicates a failure in some synthesis. For example, if vitamin B_1 were not made, growth would not occur on the minimal medium. Strains showing growth on "complete" but not on minimal medium are systematically tested on minimal medium with supplements of known compounds and in this way classified. Whether or not they differ genetically from the original strain is readily determined by making appropriate crosses, removing ascospores in order from the hybrid asci, and classifying the cultures from them for ability to grow on appropriate known media.

Strains, each differing from the original wild strain in a single relevant gene, have been obtained, each of which fails to grow in the absence of one of the

vitamins thiamin, pyridoxin, *p*-aminobenzoic acid, pantothenic acid, inositol, nicotinic acid, and choline (137). In each it is supposed that some one gene essential to the biosynthesis of the indicated vitamins has been inactivated or eliminated. In a similar way in another series of strains each requires a supplement of one of the known amino acids arginine, lysine, leucine, valine, methionine, tryptophan, proline, and threonine (74, 137): These, too, each differ from wild type by a single gene, the normal allele of which evidently plays some essential rôle in amino acid biosynthesis. In one case a mutant strain differing from wild type by a single gene requires both valine and isoleucine for normal growth (28). It is supposed that in the synthesis of these closely related amino acids there is a common reaction controlled by a given enzyme, the activity of which is in turn dependent on a specific gene.

Other strains require purines, pyrimidines, or the nucleosides or nucleotides of these for normal growth (137, 188, 310). Loring and Pierce (188) have reported that for a particular strain uridine and uridylic acid are many times more effective in promoting growth than uracil and that, while cytidine and cytidylic acid are active, cytosine is entirely inactive. These results suggest that the biosynthesis of cytidine and of cytidylic acid does not go through cytosine, a suggestion in line with the observation that free pyrimidines are not metabolized by mammals, whereas their ribonucleosides and nucleotides are (187).

An interesting practical application of mutant strains of *Neurospora* is in bioassays for particular vitamins and amino acids. So far, procedures have been developed for bioassays for pyridoxin (299, 300), *p*-aminobenzoic acid (312, 317), choline (136, 189a), inositol (15), and leucine (240, 255). An advantage of using mutant strains over naturally occurring organisms is that there is a greater freedom of choice as to the organism used and also as to its specificity.

Still other mutant strains have been obtained in which reduction of nitrate to nitrite is genetically blocked (137). Horowitz (137) has studied two strains, one unable to utilize any fatty acid as a carbon source and another able to use saturated but not unsaturated fatty acids.

It is clear from the kinds of mutants so far found that the original assumption that genes must control many if not all enzymatic reactions is essentially correct. Apparently all that is necessary is that the conditions of selection be properly determined, and mutations can be obtained in which almost any predetermined reaction is blocked. It will be recalled that this technique was followed by Moewus in determining the relations of genes to the synthesis of motility and sex hormones in *Chlamydomonas*.

One of the most completely understood series of genetically controlled reactions in *Neurospora* is that leading to the synthesis of the amino acid arginine. Srb and Horowitz (283) investigated fifteen mutant strains, each of which required arginine or a related compound for normal growth. One of these grows only if arginine itself is supplied. Two others, genetically different from each other, grow if either arginine or citrulline is supplied. Four other strains, genetically different from one another and from the first three, have their growth requirements met by arginine, citrulline, or ornithine. The remaining eight of

the fifteen mutants were found to represent duplicate or replicate occurrences of the same gene mutations as are concerned in the first seven mentioned.

The combined biochemical and genetic interpretation of arginine synthesis in *Neurospora* is indicated in figure 6. Apparently essentially the same mechanism operates here as in the mammalian liver, as determined by Krebs and Henseleit (159). There is an arginase found in *Neurospora* which splits arginine to ornithine and urea. With the intervention of urease, the urea is further degraded to carbon dioxide and ammonia. It is evident that the one-gene-one-reaction concept applies to this series of reactions, especially when it is recalled that the conversion of ornithine to citrulline is postulated on independent grounds to involve two steps.

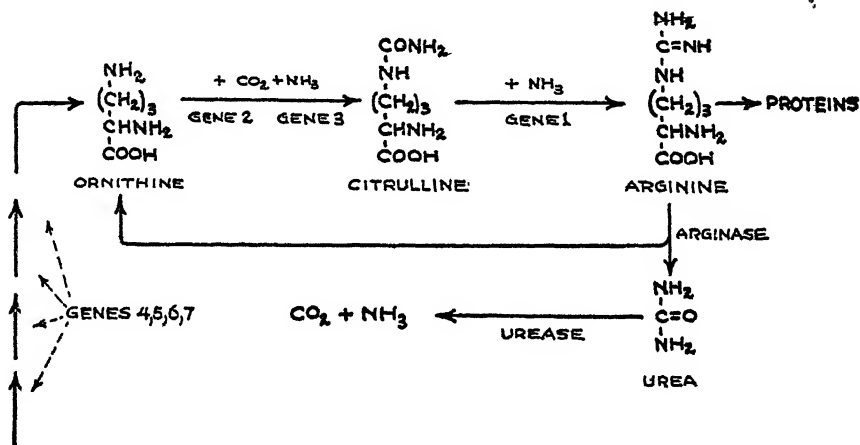


FIG. 6. Arginine cycle in *Neurospora* (after Srb and Horowitz (283))

Somewhat analogous relations are found in the synthesis of tryptophan in *Neurospora*. Here, however, the precursors were not so certainly known as in the case of arginine. Tatum and Bonner (313) have demonstrated that the final step in tryptophan synthesis involves the condensation of indole and serine, and that the serine in turn is made from *o*-aminobenzoic acid according to the reactions indicated in figure 7. A mutant strain is known which cannot convert anthranilic acid to indole. In the presence of a minimal amount of indole or tryptophan for growth, such a strain accumulates anthranilic acid and excretes it into the culture medium (314). In fact, it was this accumulation of the precursor of indole that led to its identification. Other mutant strains are known in which anthranilic acid is not synthesized. They are able to grow normally if either anthranilic acid, indole, or tryptophan is supplied.

In the case of indole formation from anthranilic acid it is clear how a genetic method can become a powerful tool in investigating metabolism. The inactivation of specific genes is equivalent to the chemical poisoning of specific enzymes, with the important difference that genes are highly specific, whereas enzyme poisons are often discouragingly non-specific. The genetic method is now being

applied in the study of several synthetic processes; for example, Horowitz (137) is studying two genetically distinct mutants that require choline or a related compound for normal growth. In the presence of a small amount of choline, one of these accumulates a choline precursor which it cannot itself use effectively but which the second strain can use quite readily, by converting it to choline. The first strain carries the synthesis up to substance X but cannot continue because of a defective gene which presumably results in a defective enzyme. In the second strain, this gene and the corresponding enzyme are active and the series of reactions can proceed normally from X to choline.

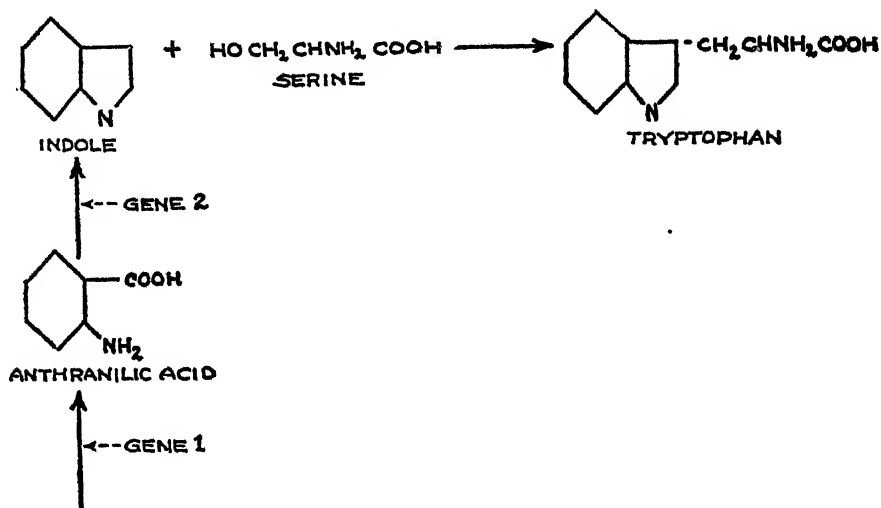


FIG. 7. Tryptophan synthesis in *Neurospora* (after Tatum, Bonner, and Beadle (314))

That different genes concerned with a series of reactions leading to a particular end product control different steps in the process is illustrated in fusion strains in which a given mycelium contains a mixture of two or more genetic types of nuclei. If two genetically different strains, each of which requires nicotinic acid or a related compound for growth, and both of the same sex, are placed together on an agar medium in which no nicotinic acid is supplied, hyphal fusion occurs, nuclei from the two strands become intermingled in a common cytoplasm, and normal growth is resumed. This complementary action, or intracellular internuclear symbiosis, shows that each of the two mutant genes is recessive. Each type of nucleus carries a normal allele of the mutant gene in the other. One carries the synthesis up to X; the other carries on from X to the final product. Several such instances have been studied by Beadle and Coonradt (17). The net result is similar to that in interspecific symbiosis, in which each species supplies a growth factor lacking in the other (310). Such coöperation in carrying out syntheses may well be a factor in hybrid vigor and in the evolutionary origin of sexual reproduction (17).

O. Miscellaneous specific reactions

Winge and Laustsen (340) have studied the inheritance of ability to ferment specific sugars in yeast. They find that in crosses in which there is a difference in this respect, diploid hybrids have the ability to ferment, i.e., ability to ferment a given sugar is dominant over inability. This was shown to be true for sucrose, melibiose, and raffinose. Following reduction divisions in which haploid ascospores are formed, segregations were observed. The material as used by these authors, however, was not favorable for establishing whether or not precise ratios were obtained. It is nevertheless probable, as Winge and Laustsen conclude, that specific genes control the production of enzymes which hydrolyze or phosphorylate the sugars mentioned. More recently Lindegren, Spiegelman, and Lindegren (179) have reinvestigated the inheritance of ability to ferment melibiose in hybrids between *Saccharomyces cerevisiae* and *S. carlsbergensis*, and have found that in diploid strains ability to ferment is dominant over inability and that in the formation of ascospores regular segregations occur. *S. carlsbergensis*, however, was found to differ from *S. cerevisiae* in carrying dominant alleles of two genes. The active allele of either gene conditions the presence of an enzyme catalyzing the splitting of the disaccharide. It is implied by Lindegren *et al.* that only one enzyme is concerned, an interpretation at variance with the one-gene-one-enzyme concept, at least in its simpler form. An alternative view, which certainly has not been excluded and which is in agreement with most recent views on the splitting of compound sugars, is that one gene concerns the production of a hydrolytic enzyme, while the other is responsible for the specificity of an enzyme catalyzing phosphorylytic splitting of the sugar. On this basis *S. cerevisiae* would have inactive recessive alleles of both these genes.

An additional finding of Lindegren *et al.* and Spiegelman *et al.* that is of the greatest significance is that if one enzyme (possibly either one but probably a particular one) is present in the cytoplasm of an ascospore which does not carry the active allele of either gene, and if the spore and its descendants are grown in the continued presence of melibiose, the strain continues to split the sugar indefinitely (179, 281a). The relation of this observation to theories of enzyme reproduction and to mechanisms of gene action will be discussed later (page 86).

The production of the enzyme amylase in the silkworm has been reported by Matsumura to be genetically controlled (original paper unavailable to author, cited from Wright (347)). Some strains show a strong amylase activity, whereas in others it is weak. It is reported that one gene pair differentiates strong from weak races with respect to the digestive juices while a second gene, strongly linked with the first, differentiates between strong and weak races as far as the body fluid enzyme is concerned. Whether or not the two enzymes are in any way different is apparently not known. Again it seems possible that one might be a phosphorylase and the other a hydrolase.

A type of Indian corn known as "waxy" has been known for many years as a curiosity and as a useful genetic character. Waxy corn kernels have red-stain-

ing rather than normal blue-staining starch (33). This is also true of the embryo sac and pollen grain starch. In the latter the difference in starch in the two kinds of haploid pollen grains produced by heterozygous plants becomes evident after only one or two nuclear divisions of the meiotic products and after only a few days. Brink and others (33) have studied the chemical differences between waxy and normal tissues and find a difference in amylase activity. More recently it has been found that there are important differences in the physical properties of waxy and normal starch which suggest that the waxy type of starch contains a higher proportion of branched-chain molecules than does normal starch (132, 282). Here again the two alleles of a specific gene appear to impart different specificities to the enzymes elaborated under their guidance.

In white clover (*Trifolium repens*), as well as in other leguminous plants, there are inter-plant differences in cyanogenetic glucoside content. These compounds may liberate hydrogen cyanide under certain conditions and therefore be toxic to livestock. The glucosides concerned in white clover are lotaustralin and linamarin (207). Under the influence of the enzyme linamerase (56) these are split to hydrogen cyanide, glucose, and a ketone (ethyl methyl ketone and acetone, respectively). It has been shown that strains may differ genetically both in their ability to synthesize the cyanogenetic glucosides and in the presence of the hydrolytic enzyme (4, 57, 58, 337). Each is dependent on the dominant allele of a specific gene, and the two genes are inherited independently. Plants carrying the normal allele for glucoside synthesis but not that necessary for the presence of the hydrolytic enzyme have the glucoside present. Atwood and Sullivan (4) raise the question of how the glucoside can be present in the absence of the enzyme which they evidently believe should be concerned in synthesis as well as degradation. They propose that the recessive allele of the enzyme gene is not completely inactive and that therefore plants homozygous for it have sufficient enzyme to carry out the synthesis. An alternative and preferable view is that the enzyme responsible for hydrolysis is not concerned in synthesis.

A mendelian recessive trait is known in the rabbit in which the fat is yellow rather than white, as in normal rabbits (42, 230). This apparently is the result of inability to oxidize ingested carotenoid pigments which are then accumulated in the fat (338). If homozygous yellow-fatted animals are fed on a carotenoid-free diet, they have white fat. The dominant allele of the gene concerned is apparently in control of a specific enzyme which has been referred to as xanthophyllase (42).

Zechmeister and his coworkers (354) have shown that the "tangerine" and normal red tomato, known to differ in one pigment gene, contain stereoisomers of lycopene. The tangerine type contains a *cis*-isomer known as prolycopene, while the red form contains all *trans*-lycopene. In the biosynthesis of lycopene the prolycopene isomer may be an intermediate or it may be a secondary product resulting from a genetically blocked reaction.

Levy and Michel (175) observed that individual rabbits differ in the ability of their blood to hydrolyze the plant alkaloid atropine. It is known that this

reaction is enzymatically catalyzed. Sawin and Glick (259) have demonstrated that the enzyme concerned, atropinesterase, is dependent for its presence on the dominant allele of a specific gene. Ability to hydrolyze atropine is therefore inherited as a simple mendelian dominant trait.

P. Summary of specific gene-controlled reactions

A summary of the specific chemical reactions known to be gene-controlled in at least one organism is given in table 1. Cases such as those of oxidation and of xanthophyll in the rabbit and of 3,4-dihydroxyphenylalanine to melanin in various vertebrates are omitted because the products of the reactions are not known.

It is an interesting commentary on the development of biochemical genetics that twenty-four of the twenty-five reactions listed here have been related to specific genes within the last ten years, while three-fourths of them have been so related during the last five or six years.

V. CHEMICAL NATURE OF CHROMOSOMES AND GENES

During the past several years rapid advances have been made in our knowledge of the chemical and physical properties of chromatin. The literature has become substantial, but on many important points no general agreement has yet been reached. Because of this it is possible in the present review only to indicate briefly some of the directions along which progress is being made. For detailed treatment of the subject reference is made to recent reviews by Mirsky (211), Gulick (123, 124), Schultz (263, 264), and Muller (219), and particularly Volume 9 of the Cold Spring Harbor Symposia on Quantitative Biology—*Genes and Chromosomes; Structure and Organization*, which reviews pertinent work from several viewpoints.

A. Isolation and analysis of chromatin

An obvious and direct method of determining what the hereditary material is chemically is to isolate nuclei or chromatin which contain the genes and subject them to analysis. Unfortunately this is not simple, for it is certain that chromatin contains many kinds of genes and it is most probable that in addition to genes it contains much non-genic material. Furthermore, the compounds involved are not resistant to rough chemical treatment and the very properties in which we are most interested may be destroyed in the process of isolating them. Miescher, who worked near the end of the last century, showed that sperm, which are made up largely of nuclei with little cytoplasm, and pus cell nuclei contain proteins and nucleic acid. Similar methods were used later by Kossell and by Levene. It is now known that the methods used by all of these early workers were much too drastic. They employed acids, alkalis, and heat and certainly greatly altered the physical properties of the materials with which they were working (211).

In 1924 Hammarsten began a series of studies on nucleic acid isolated from cell nuclei by simple extraction in large volumes of water. Material isolated

TABLE 1

Summary of specific reactions known to be gene controlled

In all cases, where it can be determined, the ability of the organism to carry out the reaction is inherited as a dominant trait

SYSTEM OF REACTIONS	REACTION	ORGANISM	AUTHORITY	REMARKS
Anthocyanins and related pigments . . .	Cyanidin → pelargonidin	<i>Callistephis</i>	Wit (341)	
		<i>Streptocarpus</i>	Lawrence <i>et al.</i> (172)	
	Cyanidin → delphinidin	<i>Lathyrus</i>	Beale <i>et al.</i> (21)	
		<i>Callistephis</i>	Wit (341)	
		<i>Streptocarpus</i>	Lawrence <i>et al.</i> (172)	
	Anthocyaninidin-3-glycoside → 3,5-glycoside	<i>Verbena</i>	Lawrence and Price (170)	
Quercetin-3-glucoside → cyanidin-3-glucoside	<i>Zea</i>	Sando <i>et al.</i> (257)		
Tyrosine metabolism	2,5-Dihydroxyphenylacetic acid → acetoacetic acid	Man	Garrod (101)	
	Phenylpyruvic acid → <i>p</i> -hydroxyphenylpyruvic acid	Man	Fölling (98)	
Tryptophan metabolism . .	Tryptophan → α -oxytryptophan	Insects	Butenandt <i>et al.</i> (37)	
	<i>o</i> -Aminobenzoic acid → indole	<i>Neurospora</i>	Tatum <i>et al.</i> (314)	
Arginine synthesis	Ornithine → citrulline	<i>Neurospora</i>	Srb and Horowitz (283)	
	Citrulline → arginine	<i>Neurospora</i>	Srb and Horowitz (283)	
Purine metabolism	Uric acid → allantoin	Dog	Trimble and Keeler (321)	
Thiamin	Thiazole + pyrimidine → thiamin	<i>Neurospora</i>	Tatum and Beadle (311)	
Pantothenic acid	Pantoyl lactone + β -alanine → pantothenic acid	<i>Neurospora</i>	Tatum (310)	

TABLE 1—Continued

SYSTEM OF REACTIONS	REACTION	ORGANISM	AUTHORITY	REMARKS
Carotenoid pigments.....	Protocrocin → crocin + gynotermone	<i>Chlamydomonas</i>	Moewus (214)	Enzyme active only in light
	Gynotermone → androtermone	<i>Chlamydomonas</i>	Moewus (214)	
	<i>cis</i> -Cronin → <i>cis</i> -dimethyl crocetin	<i>Chlamydomonas</i>	Moewus (214)	
	<i>trans</i> -Crocine → <i>trans</i> -dimethylcrocetin	<i>Chlamydomonas</i>	Moewus (214)	
	Polycopene (<i>cis</i>) → lycopen (<i>trans</i>)	Tomato	Zechmeister <i>et al.</i> (352)	
Carbohydrate splitting*....	Sucrose → fructose + glucose	Yeast	Winge and Laustsen (340)	Enzyme inferred
	Melibiose → glucose + galactose	Yeast	Winge and Laustsen (340)	Enzyme inferred
	Raffinose → fructose + melibiose	Yeast	Winge and Laustsen (340) Lindgren <i>et al.</i> (179)	Enzyme inferred
Cyanogenetic glucoside hydrolysis.....	Lotaustralin → ethyl methyl ketone + HCN + glucose	Clover	Corkill (58) Atwood and Sanford (4)	Enzyme <i>in vitro</i>
	Linamarin → acetone + HCN + glucose	Clover	Corkill (58) Atwood and Sanford (4)	Enzyme <i>in vitro</i>
Atropine hydrolysis.....	Atropine → tropine + tropic acid	Rabbit	Sawin and Glick (259)	Enzyme <i>in vitro</i>
Nitrate reduction.....	Nitrate → nitrite	<i>Neurospora</i>	Horowitz <i>et al.</i> (137)	

* Not determined for certain whether reaction is hydrolysis or phosphorolysis.

in this way was studied by several investigators (review, 211) and shown to be a highly polymerized fibrous material, the particles ranging from a molecular weight of 200,000 to over 1,000,000. This nucleic acid is known to contain the pentose desoxyribose and is commonly known as desoxyribonucleic acid. X-ray

diffraction analysis shows that desoxyribonucleic acid fibers have strong periods along their longitudinal axes at intervals of 3.34 Å. This spacing is almost exactly the same as that of the amino acid residues in a fully extended polypeptide, a fact of significance in the union of nucleic acids and proteins through salt and other linkages. The purine and pyrimidine nucleotides (base plus sugar plus phosphoric acid) are assumed to lie in planes perpendicular to the fiber axis.

Mirsky and Pollister (212, 213) have recently developed a method of isolating intact nucleoproteins from nuclei. The nuclei are first separated from the remainders of the cells in any of several ways (review, 211). In isolating the nucleoproteins, the chromatin threads themselves may first be isolated (213), or the extraction may be made from intact nuclei. The procedure is dependent on the fact that the nucleoproteins of the nucleus are soluble in 1.0 *M* neutral sodium chloride solution. They are precipitated in neutral physiological saline (0.14 *M* sodium chloride for birds and mammals) as a strongly fibrous material, showing birefringence of flow. So far as can be determined, the nucleic acid component of nuclear nucleoproteins is the same from different organisms, although the criteria by which this is determined are limited. The protein component, however, may be more than 90 per cent protamine from certain fish sperm, or almost entirely histone from other nuclei. Curiously, the protein from salmon sperm appears to be largely protamine, while that from the erythrocyte nuclei of the same organism is largely histone (211). Mirsky and Pollister have made the significant observation that their preparations of histone contain no tryptophan, while those of protamines do. Protamines, in contrast to histones, do not contain tyrosine (211).

The nucleic acid of nucleohistones and nucleoprotamines is rather easily separated by treatment with strong salt solution or by dialysis. However, the nucleoproteins migrate as single units in an electric field.

That chromosomes are composed mainly if not entirely of histone or protamine-nucleoproteins as argued by Mirsky and Pollister is denied by others. Mayer and Gulick (202), for example, claim to have isolated from veal thymus gland nuclei a sulfur-rich protein and a globulin in addition to nucleohistone. Stedmann and Stedmann (292) have gone even further in claiming that the characteristic protein of chromatin is neither protamin nor histone but a newly recognized protein rich in both basic and dicarboxylic amino acids which they call chromosomin.

In summary it seems fair to say that the direct chemical attack tells us that chromosomes contain substantial amounts of nucleoprotein made up of a highly polymerized desoxyribonucleic acid and either a protamine or a histone. The relation between protamine and the histones which apparently are found in the nuclei of different cells of the same animal is not entirely clear. Nor is it certain what other types of protein, if any, are present in chromosomes, what their relative quantities are, and whether or not they are combined with nucleic acid. On the basis of the observation that apparently *either* protamine or histone alone can be present in chromatin, it seems improbable that either is responsible for the characteristic properties of genes, although Mirsky and

Pollister (213) are inclined to believe that histones are capable of playing this important rôle.

B. Physical properties of chromosomes

It is possible to observe chromosomes in the living cell and even to manipulate them with microneedles. In this way, for example, salivary gland chromosomes of *Diptera* are seen to exhibit a characteristic stickiness, to show reversible swelling and shrinking in response to changes in osmotic concentration of the medium in which they are observed, and to be capable of being reversibly stretched to double their normal lengths (34, 35).

Attempts have been made to study chromosome structure by means of the x-ray diffraction methods so successfully used in the study of various protein fibers by Astbury and others, but technical difficulties have so far prevented definite conclusions from being arrived at in this way (35).

C. Staining reactions

The classical methods of studying chromosomes under the microscope have involved the use of basic dyes. While these, especially as applied to the giant salivary gland chromosomes of the *Diptera*, have contributed much to our understanding of the behavior of chromosomes during cell division, they are in general not sufficiently specific to lead to definite conclusions regarding chemical composition.

A significant advance was made in 1924, when Feulgen and Rossenbeck (96) developed the so-called Feulgen reaction as a specific test for the nucleic acid of the nucleus. This reaction was shown later by Levene to be specific for desoxyribose. Since this pentose appears not to occur except as a component of nucleic acid, the Feulgen reaction is specific for desoxyribonucleic acid. The related ribonucleic acid, found in the nucleolus and in the cytoplasm, does not give the reaction. Desoxyribonucleic acid was soon shown to be present in the cell nuclei of all organisms, both plant and animal (211), and even in bacteria (153, 247), which by many had been thought not to contain chromatin. The desoxyribonucleic acid is confined to the chromosomes of the nuclei of higher organisms and to what appear to be homologous bodies in bacteria. In the giant salivary gland chromosomes the nucleic acid is largely confined to definite transverse bands.

It is unfortunate that no reaction is known that is as specific for ribonucleic acid. Because of this, less direct methods of detecting this analogous compound must be resorted to and in the presence of large amounts of desoxyribonucleic acid this becomes technically most difficult.

Recently Calvin, Kidani, and Goldschmidt (39a) have applied various reagents to salivary gland chromosomes in an attempt to gain information on their structure. While the results of these attempts are most fascinating, they have not been interpreted in a manner acceptable to many cytologists (e.g., Painter (227)), and accordingly, the interested reader is referred to the original papers.

D. Ultraviolet-absorption methods

Following earlier work of Kohler, Lucas, and Stark, and others (review, 211) in which the quartz microscope was used for photographing chromosomes with ultraviolet light, Caspersson (41) developed extremely sensitive methods for determining nucleic acid distribution in cells. These methods depend on the fact that the pyrimidine and purine rings of nucleic acid contribute to a characteristic absorption in the ultraviolet with a strong maximum at 2600 Å. They do not distinguish between the ribose and desoxyribose types. Apparently the purines and pyrimidines are largely confined to nucleic acid in most cells, although it is well known that the striated muscle cell is a marked exception in this regard. Since the ultraviolet-absorption technique is most useful in conjunction with both the Feulgen reaction and enzyme digestion, a discussion of conclusions supported by it is deferred until the enzyme digestion method is presented.

E. Enzyme digestion

Theoretically it should be possible to determine what materials go to make up chromosomes by treating these bodies with specific enzymes. This was appreciated by Caspersson, and he combined this method with that of ultraviolet absorption. For example, in studying the salivary gland chromosomes, ultraviolet absorption indicates accumulation of nucleic acid in the transverse bands that stain dark with basic dyes. If the protein component of the chromosome is digested with trypsin and the nucleic acid precipitated with lanthanum so as to remain in place, the bands now composed of nucleic acid only remain. Mazia and Jaeger (203), Schultz (264), Frolova (100), and others (211) have extended this method, making use of trypsin, pepsin, papain, phosphatase, nucleases, and preparations of other enzymes. The continuous framework of the salivary gland chromosome seems to be a protein, possibly of the histone type. Digestion with trypsin destroys the continuity, while pepsin, which does not break histone down completely, does not (41, 204). The continuous structure remains after removal of nucleic acid by nuclease digestion according to Mazia (204), and can be stained with ninhydrin, but Schultz (264) reports that under certain conditions salivary gland chromosomes are almost completely digested by crystalline ribonuclease.

There are several possible obvious sources of error in using the enzyme digestion method in studying chromosome structure. Some of these have been recognized but others apparently have not, at least by certain investigators. A most serious difficulty is that of being sure an enzyme preparation is pure. For example, crude nuclease preparations so often used evidently contain several known enzymes and possibly others not known (204).

F. Conclusions

It appears from the evidence obtained by the various methods indicated that chromosomes are made up of a protein framework along which nucleic acid is combined at intervals, possibly by salt-like linkages. The nature of the protein

framework is not known. It is supposed by some to be histone, but this view raises the question of what constitutes the framework in those chromosomes containing protamine rather than histone (211). Possibly other proteins are present which provide for the longitudinal continuity.

The cumulative evidence indicates nucleoprotein as the principal component of genes. Recent work on the chemical nature of viruses, which have several important properties in common with genes, lends support to this inference (page 83). The protein component may be histone or some other protein. It is probable that gene specificity is determined by the protein component. There is, however, recent evidence from work on the nucleic acid of pneumococci suggesting that this component may possibly play a part in determining the specificities of individual genes (6).

The part played by nucleic acid in gene and chromosome duplication remains a mystery, in spite of the fact that definite cycles involving this component are known to be correlated with cell division and other phenomena of genetic importance. During prophase there is a strong increase in desoxyribonucleic acid in the nucleus and a concomitant decrease in ribonucleic acid in the cytoplasm. This phase of the cycle is completed at metaphase, when the chromosomes are heavily charged with desoxyribonucleic acid and the cytoplasm almost devoid of the ribose analogue. During anaphase and telophase the cycle is reversed, with a maximum of ribonucleic acid in the cytoplasm and a minimum amount of its desoxyribose relative in the nucleus at the resting stage (review, 227). The nucleolus, a spherical body within the nucleus containing ribonucleic acid, shows a negative correlation with the chromosomes in nucleic acid content. During the resting stage it is heavily charged with ribose-type nucleic acid but disappears during active division (211). It appears from these and other observations (227) that ribonucleic acid acts as a storage form which can be drawn upon during cell division or at other times of active protein synthesis. How the transformation from one type to the other is accomplished chemically is not known, however.

Certain regions of the chromosomes in most species are "heterochromatic." These regions are heavily charged with desoxyribonucleic acid at certain stages and are consequently stained heavily with basic dyes. They have been assumed to be genetically "inert," in the sense that ordinary genes are not found to be carried in them, but recently Mather (201) has suggested that they may be important in carrying "polygenes" (see page 80). Evidently they have some important part to play in desoxyribonucleic acid economy but precisely what this is is not known (reviews: 263, 264).

VI. GENE MUTATION

A. Spontaneous changes

The frequency with which genes undergo spontaneous mutation varies both with the organism and with particular genes within one organism. Muller has pointed out that the rate at which lethal mutations arise in the X chromo-

some of *Drosophila* per unit time is sufficient to produce lethals in a high proportion of the X chromosomes in man during the course of one generation. Obviously no such rate occurs. Evolutionary flexibility demands that the rate be appreciable, but natural selection under fixed environmental conditions tends to hold it down. The equilibrium will be expected to be reached on the basis of generations as units rather than absolute time. Assuming that genes vary chemically, they must vary in their stabilities and hence be differentially subject to change through inevitable changes in their surroundings.

The temperature coefficient for lethal mutations in *Drosophila* has been estimated for temperatures from 8° to 31°C. Because of the low rates the measurements are subject to a large error, but they indicate a temperature coefficient of about 5 (review, 234). Temperature shocks (treatments for short times at temperatures below or above those at which the organism is capable of developing normally) appear to increase mutation rates significantly (234).

As has already been mentioned (page 22) genes are known which influence the spontaneous mutability of other genes. These may be general in their effects (14, 199, 234, 306), or they may specifically affect certain other genes (242). It is presumably on the variability resulting from such mutation-influencing gene changes that natural selection acts to keep the over-all mutation rate at a favorable level.

For most individual genes the spontaneous rate of mutation is so low as to make it almost impossible to measure experimentally except in particularly favorable cases. Haldane (128) has calculated that the normal allele of the gene controlling hemophilia in man must mutate to a defective allele approximately once per 50,000 X chromosomes in each generation. For various endosperm characters in maize in which large numbers can be obtained readily, Stadler (284, 285) has observed mutation rates for various genes ranging from one or less to five hundred per million chromosomes per generation. In the case of the so-called mutable or unstable genes, of which several examples are known (71), the mutation rate is much higher. Often thousands of mutant changes occur in a single organism during the course of its development.

In connection with spontaneous mutation rates it should be pointed out that these are usually from the normal allele to an allele that can be regarded as inactive or defective in some way. Actually it is experimentally almost impossible to tell, except by waiting for back-mutation to occur, whether a mutation that inactivates is a true gene change, with the gene retaining its power of self-duplication, or a complete physical loss (191, 284).

B. Induced changes

While there can be no doubt that the discovery by Muller in 1927 that mutations in *Drosophila* can be increased many fold by treatment with x-rays represents one of the outstanding achievements of genetics, it is nevertheless true that we still cannot say precisely how the effect is brought about. X-rays, gamma rays, neutrons, and ultraviolet radiation are all capable of delivering energy to the gene in a manner capable of causing mutations. X-rays and

gamma rays produce their effects through the mediation of fast electrons from atoms in the tissue. These produce ions as they pass through cells. In neutron treatment protons have a similar effect, except that because of their greater mass the ionizations along the particle path are denser than in the case of electrons. In the absorption of a quantum of ultraviolet radiation by a cell, less energy is involved than in an ionization and it seems quite clear that the effect of this type of radiation is different in other respects from that of ionizing particles.

Within the x-ray spectrum, gene mutations are apparently strictly proportional to dosage and are independent of wave length and intensity (reviews: 68, 94, 218, 319). This means that single ionizations must be responsible for the change, for otherwise there would have to be wave-length and intensity effects. On the other hand, it is not certain whether the effective ionization has to occur in precisely the molecule or group of molecules in which the final effect is brought about or whether it is possible for the necessary energy to be transferred from the site of absorption to the locus of action (reviews: 94, 99, 218, 319).

The magnitude of the effect of radiation may be great. Muller (218) points out that with a treatment of 10,000 r-units the frequency of lethals in the X chromosome of *Drosophila* is increased about 100-fold over that occurring spontaneously in one generation of 10 days. Since this treatment may be applied in a period of an hour or less, the increase per unit time may be as high as 35,000-fold. As with spontaneous mutations, most mutant changes induced by x-rays are lethal, i.e., the organism cannot develop when homozygous for them. An important question concerns whether the proportions of types of mutant changes is the same with x-ray treatment as those found naturally. If gross chromosome aberrations such as translocations (mutual exchange of non-corresponding chromosome segments) and inversions (reversal of gene sequence in a chromosome segment) are included, it is clear that the proportion of types is not the same as that for spontaneous mutations, for x-rays produce a relatively greater increase in chromosome aberrations than in gene mutations.

Many x-ray lethals are found on cytological examination to be small deficiencies (small segments of a chromosome removed with the broken ends re-joined). This fact raises the question of whether the sole effect of ionizing radiation is to remove genes completely (284, 285). Small deficiencies are known to produce phenotypic results similar to if not identical with gene changes which supposedly do not involve loss (191). In *Drosophila* the answer seems to be clear that at least some x-ray mutations are not losses, because "back-mutations" can be induced (319). In maize, on the other hand, the answer is not so clear. In a special test, Stadler (285) found no x-ray mutants of *A* to *a* that gave fully viable plants in the homozygous state. Furthermore, he was unable to induce mutation from *a* to *A*, using an *a* allele known to be subject to back-mutation in the presence of the *Dt* gene (page 22), in a population sufficient to give almost a million *A* losses under similar treatment (286).

Ultraviolet, on the other hand, clearly gives rise to mutations in maize similar

to those that occur spontaneously. Many *A* to *a* mutants induced in this way appear to be fully viable in the homozygous state (285).

Unlike x-rays, ultraviolet shows a strong wave-length effect in producing mutations. The curve of effectiveness in producing mutations against the wave length is sufficiently similar to the absorption spectrum of nucleic acid over the same wave lengths as to support strongly the view that this substance is closely associated with genes if not indeed a component of them (134, 135, 152, 258, 289).

Unlike gene mutations, gross chromosome aberrations do not show a linear increase in frequency with increasing dosage of x-rays, and their frequency is little if at all increased by ultraviolet treatment. The situation appears to be that these changes (translocations, inversions, and long deficiencies) require the simultaneous occurrence of two breaks in the chromosome.

If there were no complicating factors, the frequency of these would be expected to go up as the square of the dose (218). It seems, however, that these breaks do not remain available for rearrangements longer than a certain time. In *Drosophila* sperm this appears to be until they fertilize the egg, and therefore no time-intensity factor need be considered when sperm are treated (218). In *Tradescantia* (spiderwort) microspores, on the other hand, breaks heal within a matter of minutes (173, 260, 261) and complications are introduced unless time of treatment is kept constant in experiments in which dosage is varied.

In the production of chromosome breaks such as are concerned in gross chromosome rearrangements, apparently several ionizations are required for a single break (108, 173). Lea and Catcheside (173) have recently calculated that something of the order of seventeen ionizations induced in a chromatid (one unit of a divided but not yet separated chromosome) as an ionizing particle traverses it (diameter ca. 0.1μ) are required to break it. This leads to the prediction that x-rays of different wave lengths will vary in their effectiveness in inducing breaks. The fact that experimental tests show excellent quantitative agreement with predictions (44) lends strong support to their arguments.

Swanson (309) has shown in a striking manner that the ultraviolet effect is different from that of ionizing particles. If x-ray treatments are preceded by ultraviolet treatment, the x-ray effects on chromosome aberrations are partially suppressed. According to Swanson, the ultraviolet probably produces a physical change in the chromosome matrix of sheath of such a nature that restitution of breaks is favored over rearrangements of broken ends.

Since it is almost certain that gene mutations involve chemical changes of some kind in the gene, it would seem most probable that these changes could be induced at will by the proper chemical treatment. Attempts to accomplish this have been many, but almost all of them have been disappointing. It is true that many reports of success have appeared in the literature but, except for those of a special type to be mentioned below, none has been confirmed. *Drosophila* eggs have been soaked in solutions of various chemicals, chemicals have been injected into young *Drosophila* larvae, and many other treatments have been tried. Some give slight effects of doubtful statistical significance

(review, 169), while others such as feeding heavy water (353), proteolytic enzymes (352), and nucleic acid (107, 218) seem to have no effect whatever. Steinberg and Thom (293) have reported positive results with nitrite treatments in *Penicillium*, but the alleged mutations cannot be demonstrated to be due to gene changes because this organism has no perfect stage. Their results have not been confirmed in more favorable organisms. Steir and Castor (298) report a permanent change in yeast cells following cyanide treatment, but no evidence has been presented that a gene change is involved. Recently Auerbach and Robson (5) have reported that allyl isothiocyanate (mustard oil) induces mutations in *Drosophila*. Further reports on this will be awaited with interest.

As a special category, the striking effects of the alkaloid colchicine on cell division should be mentioned (72). This drug has the curious property of inhibiting cytoplasmic division by interfering with the spindle mechanism while allowing the chromosomes to reproduce normally. The result is that chromosomes become multiplied in treated cells. This is a most useful tool in both theoretical and applied genetics, but there is no evidence that any change in the genes is produced by it.


The difficulty of chemically inducing gene changes is undoubtedly in part due to the difficulty of getting reagents to the gene without killing the cell in which they are carried. It is a well-known fact that the nucleus is remarkably resistant to staining by vital dyes, an indication of its general resistance to the entrance of foreign materials. It is nonetheless a reasonable hope that someone will some day discover the right trick to bring about transmutation of the gene with specific chemical treatment.

More than twenty years ago Guyer and Smith (125, 126) reported the induction of heritable changes in the rabbit by injecting anti-rabbit-lens sera into pregnant rabbits. The offspring showed eye defects which were transmitted. Several attempts to confirm this result by other workers failed, and the antibody-induced mutations were explained away by geneticists. Recently, however, Hyde (307) has repeated the Guyer and Smith experiment on an extensive scale with adequate controls and has found results essentially identical with those of the earlier workers. In interpreting this work, Sturtevant (307) suggests that, since it is probable that genes and antigens have physically corresponding specificities, it is possible that genes, like antigens, can combine with antibodies of corresponding specificities. If this were to occur, it seems possible that gene duplication would be interfered with in such a way that daughter genes would be absent or modified, i.e., mutated. Emerson (85) has attempted to test this possibility with *Neurospora*. Extracellular adaptive enzymes were obtained from culture filtrates, immune sera directed against these were produced in rabbits, and *Neurospora* cells then treated with the antibody-containing sera. Here enzymes replace the lens protein as antigens. Although the technical difficulties of obtaining known enzymes in pure form are many and have not yet been overcome, preliminary tests indicate some hope that immunological specificities may be made use of to "direct" mutations.

The transformation of types in *Pneumococcus* is a case in which directed mu-

tation seems to have been accomplished. It has been known for some time that if avirulent rough (no polysaccharide capsule) mutant forms of one type are allowed to back-mutate to a virulent smooth form in the presence of an extract from a virulent smooth form of another type, the back-mutation is associated with a change of type to that of the form supplying the type-directing substance. In the absence of such a directing agent the reversion of rough to smooth is invariably brought about with no change in type; that is, the rough reverts to a smooth of the same type as the smooth from which the rough was originally obtained. Avery, MacLeod, and McCarty (6) have recently isolated the directing agent in pure form and found it to be a nucleic acid. By every available test the activity of this is due to nucleic acid and not to some impurity present in undetectably small amounts. The activity is very high and a small amount of this specific nucleic acid causes the reverted type-changed strain to make more type-specific nucleic acid like that supplied. This may mean that a specific gene has been mutated, or that the type-specific nucleic acid is capable of autocatalytic reproduction. The latter alternative amounts to essentially the same thing as the suggestion of Wright (349) that the nucleic acid itself may be the gene and that the transformation of type is brought about not by mutation but instead by actual transfer of the gene.

Unfortunately, pneumococci do not reproduce sexually, so no direct test can be made to determine the relation of type-change alterations to gene mutations as known in other organisms. This difficulty applies as well to bacterial variations induced in other ways (70, 119, 250a).



VII. GENE ACTION

A. Interaction of alleles

In diploid organisms a mutant allele of a given gene is called recessive if an individual heterozygous for it is phenotypically like the form homozygous for the original allele. If the heterozygote is like the homozygous mutant form, the mutant is said to be dominant. These terms are convenient but obviously have little real significance, since all degrees of intergradation are found. Thus we refer to phenylketonuria in man as a recessive trait, because heterozygotes seem to be indistinguishable from normals. But, as Penrose (cited from Haldane (130)) has indicated, it appears on detailed study that such heterozygotes are more susceptible to senile dementia than are homozygous normals. In many other cases as well, recessive mutants turn out to be only partially recessive, i.e., intermediate, when a sufficiently refined study is made.

Objective and quantitative measures are needed of the effects of different alleles of a given gene in terms of rates of specific reactions or amounts of specific products formed. While this has been recognized by many geneticists (e.g., 110, 347), very few data are available on which quantitative analyses can be made in such terms. This is in part due to the fact that until relatively recently the number of specific chemical reactions known to be gene controlled has been limited. The most detailed attempt to develop quantitative interpre-

tations of dominance has been made by Wright (344, 346, 347, 348) in connection with melanin formation in the guinea pig. Here there is an obvious limitation, in that the specific reactions by which melanin is formed are not known. In fact, it is not even known precisely what melanin is chemically. In spite of these limitations Wright has been remarkably successful in developing a formal interpretation in terms of theories of gene action which he has formulated.

In the transformation of a substrate to a product genes can obviously play a part in several ways. One of the simplest is that in which the gene is inactive and produces no enzymatic catalyst whatever or an inactive one. Such an allele is known as an *amorph*. The gene-controlled catalyst (the gene might conceivably itself act directly as a heterocatalyst) may be only partially inactivated (a *hypomorph*), in which case the dominance relations will depend on the degree of inactivation, the concentration of substrate, and the reaction coefficients. A gene change may alter the catalyst in such a way that an altered product is formed with a modified efficiency in the production of the final character. If the product is entirely ineffective, the mutant allele would be an *antimorph* in Muller's terminology. If it were of low efficiency relative to the normal product, the mutant allele would be a *hypomorph* when compared with an *amorph* but an *antimorph* when compared to an allele of higher efficiency. It is also theoretically possible (349) that a gene-controlled enzyme may catalyze the transformation of two different substrates. An altered enzyme produced under the guidance of a mutant allele of the gene might have its efficiencies differentially altered with respect to the two substrates. Carried to an extreme, this type of modification might lead from action exclusively on one substrate to similar action on a different substrate, in which case the mutant allele would be classified as a *neomorph*. For several of these possibilities Wright (346, 347) has formulated the theoretical considerations in mathematical terms; the reader is referred to his papers for details.

Stern and his coworkers (295, 296, 297) have presented an interpretation of the action of alleles of a gene in *Drosophila melanogaster* having to do with the details of wing venation and certain other characters. This involves the assumption of two variable properties of the gene (or the enzyme controlled by it): namely, (1) efficiency of transforming substrate to product and (2) combining power of gene or enzyme. While this interpretation is at present rather highly speculative, it is just such treatments that state fundamental biological problems in ways in which they can be attacked profitably at a chemical level.

It is probably true that most genes are capable of existing in several forms. Certainly this is so in many particular instances (294). In such cases it is usually found that the several alleles affect the same final character in ways that can be ascribed to quantitative differences in the efficiencies of the alleles or the enzymes they control. For example, in the white series of alleles for eye color in *Drosophila* in which more than a dozen members are known, all alleles are related to pigment formation and can apparently be arranged in a series according to their efficiencies in promoting this process. The pigments produced in the presence of different alleles vary in their absorption spectra,

possibly owing to different degrees of condensation of identical pigment molecules (91). In terms of action, they presumably produce enzymes of different catalytic efficiencies or different amounts of the same enzyme.

In genes determining antigen specificities, several alleles with differing specificities are often known, as has already been pointed out for the A-B blood group gene in man, for the *Rh* gene in man, and for several others. The self-sterility alleles are another example of a similar phenomenon.

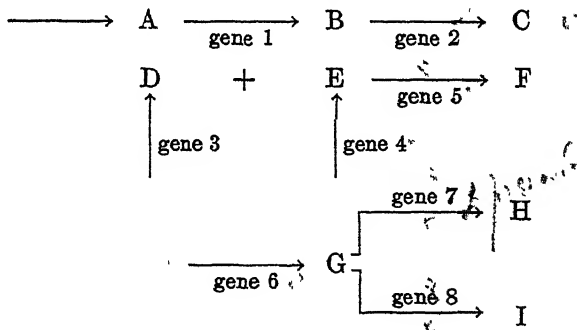
There are several instances in which a series of multiple alleles show independent variability in two or more properties. This is so for the *P* gene in maize, which is concerned with both cob and pericarp colors (red or brown water-insoluble pigments of unknown chemical nature). Here the effects on the color of the cob and that of the pericarp (outer layer of the kernel derived from maternal tissue) vary independently (2) and no simple series can be made covering all effects. A similar relation is found in the alleles of the *A* gene in maize (83, 242, 285), where the effects are: (1) on anthocyanin pigments in the aleurone (endosperm cell of the kernel) and other parts of the plant and (2) on pericarp pigment. In the *R* series of alleles of maize Stadler and Fogel (287, 288) conclude that there are many alleles and that they can be classified in terms of three independent effects as follows: (1) on aleurone anthocyanins, (2) on anthocyanins of seedling plant, leaves, husks, glumes, and others, and (3) on anthocyanin pigment in the pericarp. They suggest that even more effects may be involved. These three effects vary both quantitatively and qualitatively for different alleles. At least two of the gene effects are subject to independent modification through mutation. An allele promoting both aleurone and plant color may mutate to an allele with either of these effects reduced or lost, but usually both are not lost together (285). A series of alleles of a gene that plays a part in anthocyanin distribution somewhat like that of the *R* series in maize is known in cotton (267).

Genetically the determination of whether two hereditary units are alleles of the same or different genes are made on the basis of (1) their interaction in heterozygotes (a/a' vs. a/A a'/A') and (2) whether or not they show crossing over with one another. In the great majority of cases these two criteria agree with each other and give a clear answer. But there are instances in which either or both criteria break down. For example, bar and infrabar are two dominant genes in *Drosophila* affecting the shape of the eye. On the basis of interaction they appear to be alleles, but occasionally they cross over with each other, indicating that they are separate genes (305). Recently similar relations have been found at two other loci in *Drosophila*, one involving recessive alleles of the lozenge gene (225) and the other the dominant character star (176). In two of these cases (bar and star) it has been shown by cytological examination that the original mutant type arose as a result of a duplication of a small section of chromosome inserted in the chromosome next to the original segment with which it is homologous. On this basis the cross-over results find a ready explanation (176). The lozenge example cited may be similar, and no doubt still other situations of the same general nature exist in many organisms.

At the scute locus in *Drosophila* there exist gene forms recessive to their wild-type alleles which interact when put into the same individual to give wild type (32, 75). This suggests that they are non-allelic. However, no crossing over occurs between them, and both are alleles of a third gene form by the two indicated genetic criteria. A case of spurious allelism with at least a superficial resemblance to the scute case has been shown to arise in maize as a result of very small deficiencies produced by a special technique (191). This has been interpreted on the basis that one deficiency involves one gene, a second non-overlapping deficiency an adjacent gene, and a third deficiency both genes. By the genetic test the two short deficiencies are not alleles of each other but are both alleles of the third deficiency. The same situation would arise if recessive genes were substituted for the two small deficiencies. In this model case the deficiencies are so small that, except for particularly favorable circumstances, they could not be seen in cytological examinations.

B. Interactions of non-allelic genes

With gene-controlled reaction systems such as the following:



most of the types of interactions of non-allelic genes observed by geneticists find ready interpretation in terms of changes of normal genes to amorphic, hypomorphic, or antimorphic alleles. For example, genes 1 and 2, or 4 and 5 are complementary. In the diploid offspring of a zygote heterozygous for amorphs for either pair, segregation would give a 9:7 ratio. Genes 2 and 5 or 7 and 8 are independent and the corresponding F_2 phenotypic ratio in a diploid is the familiar 9:3:3:1. Genes 6 and 7 or 6 and 8 show an interdependence such that the expression of 7 or 8 is dependent on the activity of 6. This relation is often spoken of as epistasis, i.e., gene 6 is epistatic to 7. If antimorphic mutant alleles are concerned, the other genetic ratios result. All of these types are known in terms of specific reactions. The $A \rightarrow B \rightarrow C$ sequence is represented in the ornithine cycle (page 61). The system in which D and E, each dependent on gene activity for its formation, react to give F is illustrated in the formation of tryptophan through the condensation of serine and indole or the union of pyrimidine and thiazole compounds to give thiamin. The third system, in which the formation of two products depends on a common precursor, is

represented in melanin formation (page 30), in the formation of anthocyanins and anthoxanthins (page 39), and in the production of the two pigment components of the *Drosophila* eye (page 33).

A special case, that of so-called duplicate genes, is found regularly in polyploids and occasionally in diploids. Here the presence of either of two genes permits a given reaction to take place. In polyploids it is clear that such genes with similar functions actually represent physical duplications; the two genes are structurally and functionally identical. Presumably such duplicate genes occasionally arise in haploid or diploid organisms through the occurrence of small duplications. Such duplications are probably of the greatest importance in the process of evolution in providing material from which new genes and new gene-controlled activities can be acquired by the organism as a result of subsequent neomorphic mutation.

Many characteristics of the organism, such as height in man, yield in crop plants, and others, show almost continuous variability as a result of variation in many genes, each of which contributes a small part to the total variability. Such genes are sometimes known under the special term "polygenes" (reviews: 200, 201, 270). There is, however, no need to assume their action to be different in any fundamental way from that of genes such as those which we have been considering (but see Mather (201)).

Heterosis or hybrid vigor is a phenomenon well known to plant and animal breeders. It finds its genetic explanation in the complementary action either of alleles (80, 143, 285) or of non-allelic genes (80, 144). In terms of specific syntheses it can be illustrated by examples in *Neurospora* where two strains, each deficient in the ability to make a specific growth factor, can combine to make a heterocaryon (physiological equivalent of a diploid) in which synthesis of the two essential growth factors is complete (17).

C. Position effect

It is observed in many organisms that genes perform their tasks in a normal manner regardless of their sequential arrangement in the chromosomes. That is, inversions, translocations, and other types of chromosome rearrangements do not usually result in any phenotypic change in the organism carrying them provided there is no net gain or loss in genic material. There are, however, a number of instances known in which the action of specific genes is dependent on their positions with respect to other specific genes (reviews: 73, 224) or to heterochromatin (263, 264). Several such instances are known in *Drosophila* (73, 224, 228) but only one in plants (43). In several cases the effect is reversible, i.e., the original manner of action is restored if the genes are returned to their original position. This reversibility is of course an obvious way of ruling out the possibility that the change in position was accompanied by a true gene mutation. Although several interpretations of the position effect phenomenon have been suggested (92, 224, 295, 305), none is entirely satisfactory in all respects. In most instances the position effect is observed in structural heterozygotes (individuals heterozygous for inversions, translocations, or other

chromosome rearrangements), and Ephrussi and Sutton (92) have recently suggested that physical distortion of the gene due to somatic pairing in such heterozygotes may be responsible for a change in its function. This interpretation was inspired by the recent observation that muscle myosin differs in its enzymatic activity in the extended and contracted states (63, 88). As Ephrussi and Sutton recognize, this interpretation is not completely satisfactory in all respects. Schultz (263) has offered suggestions as to how proximity to heterochromatin may alter a gene's activity, but these are at present highly speculative. Evidently an entirely satisfactory interpretation of position effect in chemical terms lies in the future.

D. Multiple effects of genes (pleiotropism)

The profound consequences to the organism of a single gene substitution have been observed in many cases, and such observations have led naturally to the view that a single gene may do several things, often apparently unrelated. The creeper fowl, studied in detail by Landauer and his associates (163), is a case in point. In birds heterozygous for the creeper mutant allele the long bones are shortened and the mature individual is a disproportionate dwarf. The homozygote dies before hatching from the egg and shows many abnormalities. As these are studied in more and more detail it is found that most of them can be referred to defective yolk-sac blood circulation, which can be detected at 54 hr. of incubation (39). If the eye of a homozygous creeper is transplanted to a normal embryo with normal circulation it develops normally (102). Other effects, however, appear to be determined earlier (251). It seems highly probable that one primary change is responsible for the creeper syndrome.

A similar situation is found in a recessive lethal in the rat studied by Grüneberg and others (86, 95, 130). Here over twenty abnormalities in development have been discerned. Careful study shows that they are all related causally and have their primary origin in anomalous development of cartilage cells. A somewhat similar situation is known in the mouse, where the syndrome of inherited hydrocephaly stems originally from defective cartilage cells (121, 122).

Grüneberg (121) has ably discussed the question of pleiotropic gene effects and comes to the conclusion that probably most if not all genes primarily influence one process that is cell- or tissue-specific. This view agrees with the notion that genes produce their effects through controlling specific reactions. These of course would be expected to be cell-specific and tissue-specific rather than organ-specific.

E. Maternal effects

It is observed in a number of instances that the effects of specific genes are evident in cells in which the determining gene alleles themselves are not present. The classical case of this is in the direction of coiling of snail shells, where the inheritance follows the usual mechanism except that the direction of coiling of a given individual is determined by the genetic constitution of its mother

rather than of itself (29, 304, 308). Direction of coiling is apparent at the first cleavage of the egg and evidently depends on the genetic constitution of the egg mother cell before reduction in chromosome number.

From the standpoint of the chemical basis of such genetic carry-over effects, Kühn and Plagge (160) have observed that eggs of the meal moth *Ephesia* which are homozygous for the *a* allele of the *A,a* gene pair carry *a*⁺ hormone which is dependent on the *A* allele for its production. This physical transfer of hormone to the offspring results in pigmentation of the larval skin and eyes. The effect decreases during development and disappears by the following generation. The hormone concerned has been shown chemically to be α -oxytryptophan or a closely related compound (page 34).

Other cases of so-called maternal influence have been summarized by Plagge (233); presumably a similar interpretation involving transfer of gene-dependent substances through the cytoplasm is involved in all of them.

F. Genes and protein synthesis

The available evidence is consistent with the hypothesis that genes are proteins or nucleoproteins and that their two primary actions lie in autocatalytic control of the synthesis of more units like themselves and in determination of the specificities of non-genic proteins. These two functions may be fundamentally one (130, 215, 217, 324). The proteins synthesized under gene guidance may be enzyme proteins, antigen proteins, or possibly structural proteins. Unfortunately there is no general theory of how genes duplicate themselves or control protein specificities that has any support in experimental observation. Since it is not even known with any certainty how a peptide linkage is formed (145), it is clear that all hypotheses having to do with this important question must of necessity be highly speculative. Perhaps the most widely held view (69, 130, 217, 219) is that the gene somehow acts as a master molecule or templet in directing the final configuration of the protein molecule as it is put together from its component parts. Delbrück (69) has advanced a speculative hypothesis as to how such a model-copy system might operate in terms of short-distance interactions between gene and appropriate component parts in such a way as to lead to an accurate copy.

Whether the component parts that are fitted together under gene direction to give the completed molecule are amino acids, amino aldehydes, dipeptides, or units of higher order, it is evident that these too must be synthesized under the direction of many genes, each controlling one specific reaction. This means that while the final configuration is determined by one model gene, many other genes take part indirectly in the reproduction of a gene or in the synthesis of a protein molecule. In general, these secondary reactions will be common to the synthesis of many other genes as well.

If the gene acts either directly as an enzyme or indirectly by controlling enzyme specificity, the latter being a necessary assumption for extranuclear enzymes, it is evident that the primary effect of a gene mutation will be on a single reaction. Many secondary effects will of course follow, as is known, for ex-

ample, in hereditary failure to oxidize phenylpyruvic acid in man. From an experimental point of view it is not always easy to determine whether or not a particular reaction interrupted when a substitution at a given gene is made is the reaction controlled by the gene in a primary way. As an example, suppose a mutant form were characterized by inability to combine pyrimidine and thiazole to form thiamin. Carboxylase, which contains thiamin pyrophosphate as a prosthetic group, would be inactivated. How would one distinguish between thiamin synthesis and formation of carboxylase protein as the primary action of the gene concerned? If administration of thiamin were to relieve the difficulty, formation of carboxylase protein would be ruled out as a primary effect. Thiamin synthesis presumably involves an enzyme catalyzing the union of the pyrimidine and thiazole moieties. It is possible that the "S" factor of Kidder (148) serves as a prosthetic group in this enzyme. If so, and if administration of "S" factor were to enable the mutant form to synthesize thiamin, the primary gene action would lie in some reaction in the synthesis of "S" factor; if not, the primary action would be in the production of the protein component of the enzyme for thiamin synthesis. Such an analysis depends on the prosthetic groups or coenzymes being obtainable for the enzymes of the reactions that might *a priori* be primary. Unfortunately, of course, this is not often the situation. ✓

VIII. VIRUSES AND PLASMAGENES

Almost as soon as the discovery of viruses became known to the world, Troland (324) appreciated that fundamentally they were very "gene-like." His vision is indicated in the following quotation taken from his paper published in 1917, two years after Twort first demonstrated filterable agents and the same year in which d'Herelle published independent confirmation:

There is considerable evidence that free autocatalytic enzymes exist in our biological universe even at the present day. Such an hypothesis would serve to account for the specific contagious diseases, such as measles, rabies, and smallpox, which have been demonstrated to possess "filterable viruses."

This quotation assumes added significance when it is pointed out that it was Troland's belief that genes and enzymes are intimately related. Since Troland's paper, the evidence for basic similarity between genes and viruses has steadily increased (217, 219). We may summarize these similarities as we know them today as follows:

✓(1) Both lie within the same size range. Viruses cover a considerable range in size (11, 290, 291). While estimates of the sizes of genes are relatively crude (218), they suggest that genes are of the same order of size as tobacco mosaic virus, a medium-sized virus.

(2) Both appear to be or at least to contain nucleoproteins. The evidence that this is so for genes has been summarized—it consists of chemical analysis of chromatin and the observation that the ultraviolet wave-length mutation spectrum parallels the absorption spectrum of nucleic acids. Purified viruses have been subjected to direct analysis and shown to be ribose nucleoproteins

with the protein components more complex than protamines or histones (11, 290, 291). Bacterial viruses likewise appear to be ribose nucleoprotein in nature (review, 70). Genes appear to contain desoxyribonucleic acid rather than the ribose analogue, but this difference is not certainly established.

(3) Both have the property of self-duplication in the proper environment, i.e., in actively metabolizing living cells of the proper genetic constitutions. A difference in the two entities is seen in the fact that genes usually duplicate themselves only once per cell generation, while viruses are not subject to this regularity in their multiplication.

(4) Genes and viruses are both subject to mutation, i.e., changes in their properties other than those permitting self-duplication. Mutation in genes has already been discussed. The results of the process in viruses (including phages or bacterial viruses) are often observed (reviews: 11, 70, 162, 196, 290). In both viruses and genes mutation is known to be a reversible process (11, 70). In viruses it is known to involve a change in immunological specificity and a change in amino acid composition in some instances (11, 155, 290, 291). Inactivation in both genes and viruses by x-rays is known to be a "single hit" phenomenon, i.e., to be brought about by single ionizations (70, 118).

(5) Both genes and viruses influence metabolic processes in ways that often appear to be similar. It is interesting in this connection that no enzyme activity is known in cell-free viruses (70).

The origin of viruses is not known with certainty in any specific case, but on logical grounds it seems most reasonable to assume, as Darlington (67) and others have, that they arise from normal cell proteins by some change analogous to mutation. Indirect evidence for this comes from the fact that there exist virus carriers in both higher plants and bacteria (so-called lysogenic strains) which show no detectable symptoms of virus presence. Salamon and Le Pelley (256) have demonstrated that all strains of King Edward potato carry para-crinkle virus, which is without detectable effect. When the virus is transferred to certain "susceptible" varieties by grafting, symptoms of disease develop. Since no way other than grafting is known of transmitting para-crinkle virus, it is probable that the agent arose in the King Edward variety itself (67). Somewhat similar situations are known in bacterial viruses. White (334) has found that most if not all Indian strains of *V. cholerae* carry a certain virus but show no apparent effects of it. All tested Chinese and Japanese strains of the bacterium, on the other hand, are lysed by this virus.

Plasmagenes are postulated self-duplicating cytoplasmic units which, like genes and unlike viruses, are normal cell constituents (79, 269). Because it has not heretofore been possible to demonstrate the existence of these units in a manner as unequivocal as those used in the demonstration of genes and viruses, most geneticists and other biologists have been skeptical as to whether such units, comparable in autonomy to genes, really exist. The evidence is now sufficient, however, to justify serious attention to the possibility of gene-like units in the cytoplasm. Actually, of course, the distinction between viruses and plasmagenes on the basis of one being normal and the other an abnormal cell

constituent may be quite artificial. Is para-crinkle virus a normal or an abnormal component of the cell of King Edward potato? Incidentally, instances of this kind suggest that viruses may be acquired by one species from another in which they are *normal* cell components. It seems most probable that there are "viruses" carried by species for which no tester strains exist. These would most certainly be classed as normal cell constituents if there were any way of identifying them which was not dependent on the production of disease.

A second distinction between viruses and plasmagenes is that one is infectious and the other not (67). This too is obviously an artificial basis of separation. Natural transmission might well depend solely on whether or not a suitable insect vector were present.

Plastids in plants are known to have a certain autonomy in their transmission as cytoplasmic units (241). Often defective plastids arise by a mutation-like process which, regardless of the genetic constitution of the nucleus, is perpetuated indefinitely through self-duplication (138). This is not to imply in any way that plastid development and functioning are independent of gene control, for genetics has shown in hundreds of cases that such a control does exist. Regarding the relation between the two factors, gene control and self-duplication of plastids, Rhoades (243) has recently described a most instructive case. In maize a recessive gene allele is known to induce frequent "plastid mutations" to a defective type. Once these have arisen under the influence of the gene concerned, they perpetuate their kind and the change that produced them cannot be reversed by replacing the mutant form of the gene with its normal allele. ✓

That plastids and their supposed precursors, mitochondria, contain self-duplicating units in some respects like genes is indicated by two other types of evidence. Claude (52) and others (76) have shown that these cytoplasmic structures contain ribose nucleoproteins. Woods and DuBuy (342) have obtained evidence that in certain types of plant variegation due to self-duplicating defective plastids, the defective agent can be transmitted from one part of a plant to another through grafting. Only one positive case of transmission was obtained, however, and because of this and the possibility of alternative interpretations, confirmation is needed before this finding can be accepted without question.

A number of instances of apparent cytoplasmic inheritance are on record. One of the most thoroughly studied of these involves the flowering plant *Epilobium* (208, 209, 210). Here reciprocal crosses between species and between different races of a single species show differences in the hybrid plants of the first generation. Behavior in back-crosses to the parents is consistent with the assumption that cytoplasmic entities transmitted through the egg are involved. It seems probable that these are in some respects like para-crinkle virus in that their effects depend on the nuclear constitution of the plant in which they are found. They are *normal* in plants of one genetic constitution but not in those of another. They differ from viruses in not being infectious under the conditions in which they have been studied.

Sonneborn (279, 280) has reported experiments on "killer" and "sensitive" } ✓

strains of the protozoan *Paramecium aurelia*, which he interprets in terms of a substance of the cytoplasm which multiplies under gene control. Animals carrying the dominant allele of the *K* gene can produce this substance (known as kappa) provided some is already present in the cytoplasm, but they cannot initiate its formation. It can be physically transferred to an animal genetically capable of making it but lacking the primer, and it will thereafter be multiplied in step with the vegetative reproduction of the animal. The hypothetical substance kappa is apparently "adsorbed" by the *K* allele of the killer gene when this is present in the macronucleus of the animal.

A situation similar in at least certain respects is found in the transformation of the types of pneumococci under the guidance of a specific nucleic acid (6, 279). Here apparently the nucleic acid supplied the bacterium initiates a process by which more nucleic acid of the same type specificity is synthesized. If the nucleic acid is a gene (349), or a plasmagene, it must be capable of direct self-duplication. On the other hand, if it is an inducer of a specific mutation, the production of more nucleic acid of the same type would be a less direct process.

Spiegelman, Lindegren, and Lindegren (281a) have reported that an enzyme concerned with the splitting of the disaccharide melibiose in yeast is formed under the direction of a specific gene (or two under their interpretation) but that, once formed and in the continued presence of the substrate melibiose, the enzyme continues to be formed in the absence of the active allele of the gene initiating its production. Apparently the enzyme is self-duplicating in the presence of its substrate. The enzyme can be thought of as a kind of plasmagene, but one of a special type, since it reproduces only in the presence of the substrate.

A partly hypothetical chemical model of self-duplication of cytoplasmic elements can be constructed in terms of what we know about the formation of certain enzymes. As is well known, pepsin is formed autocatalytically from an enzymatically inactive precursor pepsinogen (review, 223). Both enzyme and precursor are known in crystalline form and both are proteins. While presumably closely related chemically, they differ from each other both serologically and in crystal structure. If we assume a situation in which pepsin were synthesized under the direction of a specific gene, without going through pepsinogen, we would have a standard gene-enzyme relation. It is conceivable that the pepsin-specific gene could mutate to a form which, instead of directing the synthesis of pepsin, determined the production of pepsinogen. So long as pepsin remained present, there would be no selective disadvantage in such a mutation. However, if for some reason the supply of pepsin were temporarily exhausted, the organism would remain pepsin deficient. If then a small amount of pepsin were introduced into the cell, it would multiply even in the absence of the gene which originally directed its synthesis and we would have a situation analogous to that reported by Spiegelman *et al.*, except that no substrate would be required for enzyme self-duplication.

It is becoming more and more evident as our knowledge increases that genes,

enzymes, antigens, plasmagenes, viruses, and other proteins have many properties in common. It would not be surprising, then, if with slight change in structure or in cellular environment one of these should change to another. Examples are continually being found in which traditional distinctions break down. For example, the muscle protein myosin is now thought to be both a structural protein and an enzyme (63, 88).

IX. EVOLUTIONARY CONSIDERATIONS

Troland (323, 324), Alexander and Bridges (1), Oparin (226), Plunket (235), Koltzoff (156) and others have speculated on the question of how the first autocatalytic particle, the "protase" of Troland or the "protogene", arose from materials not possessing this essential property of living things. The conclusion seems almost inescapable that this most significant step in the origin of life took place in an environment in which many organic molecules had spontaneously arisen from inorganic precursors and remained intact because of absence of organisms to break them down (226). Protogenes presumably had only the property of autocatalysis, not that of heterocatalysis. If we assume that the property of heterocatalysis was acquired by protogenes in subsequent evolutionary steps, we can imagine the gradual evolution of symbiotic systems of increasing complexity in which protogenes became genes with specialized heterocatalytic functions (1). This view underlies a possible interpretation of the evolutionary development of complex syntheses in which the intermediate products serve no use. It is commonly assumed that such systems of synthesis evolve from simpler to more complex. On this basis it might be assumed that in arginine synthesis the first step is the reduction of nitrate to nitrite. Nitrite is further reduced to NH_4^+ nitrogen. There follows a series of reactions of unknown nature, leading to ornithine. This is converted to citrulline, which in turn is transformed to arginine. But for this series of reactions to evolve from the nitrate end of the series it is necessary to suppose either (1) that all the steps arose simultaneously, (2) that the ability to synthesize each compound in the series conferred a selective advantage on the organism, or (3) that the individual steps arose independently and persisted without advantage until combined by chance into a single organism. Each of these possibilities is individually highly improbable, as is any combination of them. The last implies sexual reproduction, which presumably arose late in the process of evolution of organisms. Horowitz (135a) has recently suggested that the evolution of series of reactions leading to a final product useful to the organism, or system of symbiosing genes, may have evolved backward from the final compound in a manner in which each successive step would have a selective advantage to the system. Thus, if the original protogene took arginine from the medium in which it reproduced, a mutation leading to catalysis of the reaction citrulline to arginine would have a strong selective advantage if both citrulline and arginine were present in the medium. In a similar way mutations leading to catalysis of the reactions by which ornithine is converted to citrulline would take the system of genes a step closer to independence of preformed organic molecules

and would confer an additional advantage. Each new mutation in the system would have a chance of adding a new reaction to the series.

It should be pointed out that there is nothing in the hypothesis of backward evolution of reaction systems incompatible with the occurrence of evolutionary steps in the opposite direction. The original final product in the ornithine series might, for example, have been citrulline with arginine substituted in a later evolutionary step. Whatever was the nature of the first autocatalytic system, its component parts must have been taken from the environment. If these were organic molecules of any type, the backward evolution in the series to simpler precursors would appear to be a probable process.

Since mutations in genes leading to their loss or inactivation are more frequent than the reverse process, reactions of no advantage to the organism are not expected to persist indefinitely. This means that saprophytic plants, their counterparts among animals, and all parasitic forms which find themselves continuously in the presence of certain biologically significant compounds would be expected to lose their ability to synthesize these compounds. That such evolutionary specialization has indeed taken place in many bacteria, protozoa, and other organisms is clear from the work of Lwoff (190), Knight (154), Schopfer (262), and others. Unless intermediates in the end products no longer synthesized are useful to the organism in independent ways, all steps in a given synthesis will tend to be lost eventually. In this way specialization through loss of function will be essentially irreversible. The possibility must be kept in mind, however, of the evolutionary reconstruction of a reaction system in the manner in which it originally arose.

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THE BASICITY CHARACTERISTICS OF SCANDIUM, YTTRIUM, AND THE RARE EARTH ELEMENTS

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CONTENTS

I. Introduction.....	98
II. Establishment of relative basicities.....	100
A. Theoretical considerations.....	100
B. Experimental approaches.....	107
1. Order of precipitation by soluble alkaline materials.....	107
2. Precipitation and dissolution of hydrous oxides and hydroxides.....	109
(a) Miscellaneous measurements of solubilities and solubility-product constants.....	110
(b) Measurements based upon data from electrometric titrations.....	113
(c) Summary.....	117
3. Hydrolysis studies.....	117
(a) Chemical studies.....	118
(b) Physicochemical studies.....	120
(1) Conductivities of aqueous salt solutions.....	120
(2) Hydrogen-ion concentrations in aqueous salt solutions.....	123
(3) Viscosities of aqueous salt solutions.....	125
4. Standard electrode potentials.....	125
5. Thermochemical investigations.....	127
6. Thermal decompositions of oxygen-containing salts.....	128
C. Summary.....	129
III. Separations based upon differences in basicity.....	131
A. Precipitation by alkaline materials.....	132
1. Caustic alkalis.....	132
2. Metal oxides and carbonates.....	132
3. Rare earth oxides or hydroxides: the "oxide processes".....	134
4. Ammonia.....	135
5. Organic derivatives of ammonia.....	139
B. Fractional dissolution of oxides or hydrous precipitates.....	140
C. Hydrolysis.....	141
1. Separation of cerium by hydrolysis.....	141
2. Hydrolysis of nitrites: the "basic nitrite" process.....	141
3. Hydrolysis of azides.....	142
4. Hydrolysis of miscellaneous compounds.....	143
D. Fractional precipitation by the electrolysis of aqueous salt solutions.....	144
E. High-temperature reactions.....	146
1. Fractional decomposition of fused nitrates: the "nitrate fusion" process.....	146
2. Fractional decomposition of sulfates.....	148
3. Miscellaneous thermal decomposition procedures.....	148
F. Summary.....	149
IV. Conclusion.....	149
V. References.....	150

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I. INTRODUCTION

While the term "basicity", as applied to the metallic elements and their compounds, has been employed to cover a variety of phenomena from the ease with which the free elements lose electrons under oxidizing conditions through the extent to which metal salts hydrolyze in aqueous solution to the ease with which oxygen-containing salts decompose when heated, all such phenomena are reducible, directly or indirectly, to relative attractions (perhaps largely coulombic) of the metal ions for electrons. In this respect they are, therefore, all manifestations of acid-base behavior in terms of the modern broad electronic pictures, particularly that offered by Usanovich (155a, 405a).

Since, according to this concept, basicity involves the loss of anions or free electrons, it can be said that any property which measures the tendency of an element to lose electrons or which measures the lack of attraction which an ion has for electrons in turn measures the basicity of that element or ion. That the classical concepts of basicity in terms of the reactivities of metal oxides or hydroxides with acids or of the dissociation of hydroxides in aqueous solution are merely applications of this general idea is apparent if one considers them in the light of attraction or lack of attraction existent between cations and the electrons in the oxide and hydroxide ions. Although not explicitly stated therein, this same idea is implied in early references to basicities as affinities (278, 285). In this paper, any property relating to the relative attraction or lack of attraction for electrons will be considered as related to basicity. Such an approach is particularly applicable to considerations of elements as similar in properties and as lacking in covalent characteristics as those discussed herein.

As a family, scandium, yttrium, the rare earth elements, and actinium yield the most highly basic oxides of all the trivalent elements (66, 241, 250, 274, 285, 382, 416). There is every reason to believe that, of these, actinium forms the most strongly basic oxide and that more basic oxides can be formed only by ions with lower positive oxidation numbers or larger sizes (413, 416). Because of the scarcity of actinium, little is known of its properties or of those of its compounds. This discussion will, therefore, be restricted to scandium, yttrium, and particularly the rare earth elements.

As experimental evidence for the relatively high basicities of the oxides of these elements, one can cite the ease with which even the strongly ignited oxides of all but scandium dissolve in acids (66, 79, 250, 263, 285, 305, 416); dissolve in ammonium salt solutions with the liberation of ammonia (20, 21, 45, 244, 250, 263, 343); dissolve in aniline hydrochloride solutions (233); react with such high-temperature acids as ammonium salts (5, 146, 147, 181, 351, 352, 394, 450), borax (31, 154), metaphosphate (31), and alkali bisulfate (231, 363) or pyrosulfate (11, 212); and absorb atmospheric carbon dioxide (114, 250, 263, 278, 285). To these can be added the comparatively high water solubilities and precipitation pH values of the hydrous oxides and hydroxides (64, 65, 74, 167, 222, 297, 327, 361, 389, 398), the tendencies of at least some of the oxides to slake with water (244, 250, 278, 285), the absorption of carbon dioxide by hydrous

lanthanum (12, 294, 295, 296) and neodymium (12) hydroxide sols, and the fact that hydrous lanthanum hydroxide whether moist (21, 52, 259, 286, 383) or in colloidal suspension (294, 296) reacts alkaline. In fact, the hydrous oxides and hydroxides are so strongly basic as to render determination of the rare earth elements by electrometric titration with sodium hydroxide impossible (199).

Parallel evidence for these relatively high basicities is found in the following: the slight, though measurable, hydrolysis of aqueous salt solutions containing weakly basic anions (28, 59, 60, 75, 76, 200, 203, 204, 250, 260, 360); the stable existence of a number of normal salts derived from such strongly basic anions as carbonate, chromate, acetate, and various organic anions (173, 250, 251); the high electrode potentials for the free elements (208, 240); the tendencies of the free elements to liberate hydrogen from water (274); the high heats of solution of the oxides in acids (269, 270, 271, 272, 273); and the comparatively high temperatures required for the thermal decomposition of oxygen-containing salts (433, 438, 439). On the other hand, the fact that the salts of such extremely strongly basic anions as the sulfide (250), cyanide (251, 287), azide (2, 98, 225), carbide (298), and nitrite (172, 185, 301, 373, 374) undergo ready hydrolysis indicates lesser basicities among these elements than among the alkali and alkaline earth elements.

While these elements as a group are highly basic, significant differences, particularly between scandium and yttrium, yttrium and lanthanum, and lanthanum and lutecium, exist. The excellent agreement between the theoretically predicted variations within the family and the observed variations make the family especially well adapted to discussion. Lanthanum is generally regarded as being the most basic and scandium the least, with the cerium earths being more basic than the yttrium earths and yttrium occupying a position among the yttrium earths. As a family, the rare earths have been said to be more basic than the Group IIIB elements but less so than the alkaline earth elements (241, 260, 386, 416), to be more basic than beryllium (302, 303, 305), to be less basic than magnesium (66, 350), to resemble the alkaline earth metals in basicity (79, 285), and to be more basic than magnesium but less so than the alkaline earth elements (250). Lanthanum hydroxide has been stated to be as basic as ammonium hydroxide (408).

On the other hand, some evidence for amphoterism in the positive three state of oxidation has been presented. Thus, hydrous scandium hydroxide is measurably soluble in concentrated aqueous potassium hydroxide solutions, and crystals corresponding to



have been isolated from such solutions (389). Under similar conditions, hydrous yttrium hydroxide dissolved, but the resulting solutions hydrolyzed immediately, precipitating crystalline yttrium hydroxide (389). Lanthanum hydroxide was insoluble (389). Fusion of lanthanum oxide with sodium and lithium carbonates has been stated to yield a readily hydrolyzable tetralanthanate, $\text{Na}_2\text{La}_4\text{O}_7$, and a metalanthanate, $\text{LiH}_9\text{La}_5\text{O}_{15} \cdot 2\text{H}_2\text{O}$ (9), while digestion of hydrous lanthanum

hydroxide with concentrated aqueous solutions of sodium, potassium, and barium hydroxides yielded the respective metalanthanates (9). This apparent amphoterism is to be doubted, however, since Zambonini and Carobbi (451) were able to prepare only mixtures of lanthanum oxide or hydroxide with alkali hydroxides or carbonates by a repetition of the procedures. Wunder and Schapiro (441), on the other hand, reported considerable losses in weight when didymium (i.e., praseodymium and neodymium) and erbium oxides were fused with sodium carbonate and the resulting melts boiled with water, with lanthanum oxide undergoing a slight loss.

Further evidence for amphoterism on the part of certain of the trivalent rare earth elements appears in high-temperature reactions. Thus, Beck (14) reported the formation of KNaO_2 and KPr(OH)_4 in melts prepared from potassium hydroxide and didymium oxide and indicated that oxides of samarium-gadolinium fractions containing traces of terbium dissolve in molten potassium hydroxide. The neodymium compound underwent ready hydrolysis. Zintl and Morawietz (453) reported the formation of NaLaO_2 as a result of the interaction of sodium and lanthanum oxides at 500°C . Compounds analogous to calcium aluminate have been reported as resulting from the interaction of oxides or carbonates of magnesium, calcium, strontium, or barium with rare earth oxides at temperatures above 1500°C . (187). The less basic cobaltous oxide, on the other hand, gave no reaction with either lanthanum or ceric oxide at 1300°C . (160).

The oxides of tetravalent cerium and praseodymium are somewhat more acidic in character. The difficulty encountered in dissolving in acids oxide mixtures containing relatively large amounts of cerium has been ascribed to the presence of unreactive rare earth cerates (79, 385), and other evidence for such combinations has been offered (445, 446). While Beck (14) was unable to dissolve CeO_2 and Pr_2O_3 in fused alkali hydroxides, Zintl and Morawietz (453) obtained Na_2CeO_3 and Na_2PrO_3 by the action of sodium oxide upon ceric oxide and praseodymium sesquioxide (in the presence of oxygen), respectively.

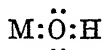
In spite of this evidence for amphoterism, it must be concluded that these elements and their compounds are predominantly highly basic in character and show acid properties only to limited extents and under unusual conditions.

II. ESTABLISHMENT OF RELATIVE BASICITIES

A. THEORETICAL CONSIDERATIONS

It has already been pointed out that variations in basicity are reducible to relative tendencies toward electron loss or gain, regardless of the property employed as a means of measuring such variations. Essentially, the greater the tendency for a material to lose electrons the more basic that material is. And conversely, once those electrons have been lost, the less the tendency for the resulting cation to attract electrons, either free or combined in anions, the more highly basic the material. As a consequence, any theoretical predictions regarding relative basicities must amount to considerations of the relative attractions and repulsions for electrons.

To illustrate the factors involved in such considerations, one may cite the hydrous oxides or hydroxides (390), the basicities of which can be measured in terms of tendencies to lose hydroxyl ions. If the presence of at least some linkages of the type



be assumed, the tendency to lose hydroxyl ions is then measured by the attraction exerted by the cation M^+ upon the electrons surrounding the oxygen. The less this attraction, the greater the ease with which the hydroxyl group is split

TABLE 1
Electronic configurations

ELEMENT	ATOMIC NUMBER	NUMBERS OF ELECTRONS													
		1s	2s	2p	3s	3p	3d	4s	4p	4d	4f	5s	5p	5d	6s
Sc.	21	2	2	6	2	6	1	2							
Y.	39	2	2	6	2	6	10	2	6	1		2			
La.	57	2	2	6	2	6	10	2	6	10		2	6	1	2
Ce.	58	2	2	6	2	6	10	2	6	10	1	2	6	1	2
Pr.	59	2	2	6	2	6	10	2	6	10	2	2	6	1	2
Nd.	60	2	2	6	2	6	10	2	6	10	3	2	6	1	2
Il.	61	2	2	6	2	6	10	2	6	10	4	2	6	1	2
Sm.	62	2	2	6	2	6	10	2	6	10	5	2	6	1	2
Eu.	63	2	2	6	2	6	10	2	6	10	6	2	6	1	2
Gd.	64	2	2	6	2	6	10	2	6	10	7	2	6	1	2
Tb.	65	2	2	6	2	6	10	2	6	10	8	2	6	1	2
Dy.	66	2	2	6	2	6	10	2	6	10	9	2	6	1	2
Ho.	67	2	2	6	2	6	10	2	6	10	10	2	6	1	2
Er.	68	2	2	6	2	6	10	2	6	10	11	2	6	1	2
Tm.	69	2	2	6	2	6	10	2	6	10	12	2	6	1	2
Yb.	70	2	2	6	2	6	10	2	6	10	13	2	6	1	2
Lu.	71	2	2	6	2	6	10	2	6	10	14	2	6	1	2

off and the greater the basicity of the material. Quite obviously, the attraction for these electrons will be governed both by the size of the cation and by the magnitude of the positive charge which it bears. The most highly basic materials would contain cations of large size and small charge, and even moderately high basicity among materials containing highly charged cations can be expected only if these cations are correspondingly large. An extension of these ideas would predict a trend through amphoterism to acidic properties as the cationic charge increased and the cationic size decreased.

An approach to the relative basicities of scandium, yttrium, and the rare earth elements through consideration of the attractions for hydroxyl groups was made by Grimm (151). Grimm pointed out that the decrease in basicity in the series lanthanum-yttrium-scandium paralleled a decrease in the molecular volumes of similar compounds of these elements and in the radii of the cor-

responding cations. He stated, furthermore, that because of a constancy in outer electronic configurations among the rare earth elements, a decrease in size should be noted among them and a parallel decrease in basicity might be expected. This contention was supported by reference to published basicity series based upon precipitation with alkalis (285).

The most comprehensive treatment of the relative attractions for electrons among these elements and the attendant effects upon properties is due to von Hevesy (411, 412, 413, 414). von Hevesy indicated that in any particular family of the Periodic Table the principal quantum number of the valence electron(s) increases as atomic number increases and that, as a result, the binding energy of the valence electron(s), or the attraction exerted by the positive nucleus, decreases. Such decreases would be apparent in the series scandium-yttrium-lanthanum, since as the electronic configurations listed in table 1 indicate, the outer configuration of each element amounts to two *s* electrons in the n^{th} quantum level and one *d* electron in the $(n^{\text{th}} - 1)$ level. An increase in heteropolar character (and in basicity) must then occur in this series.

It was further pointed out that in the elements immediately following lanthanum, i.e., the rare earth elements, the admission of successive electrons into the fourth quantum level (filling of the 4*f* orbitals) instead of into the higher levels fails to nullify the increased attractive forces between the nuclei and the outermost electrons and results in a net increase in the strengths with which these electrons are bound. In the rare earth series the attractive forces increase steadily with increasing atomic number, and the ease with which electrons are lost decreases. In the region of holmium (No. 67), the weakening of binding between yttrium and lanthanum is completely compensated for, and the electrons in holmium are held with about the same strength as those in yttrium. Elements beyond holmium hold their electrons more firmly than yttrium, but the extent of this attraction even in the last member of the series, lutecium, does not approach that in scandium. The appearance of yttrium with holmium and neighboring elements in many fractionation procedures supports these conclusions.

On the basis of these considerations, von Hevesy concluded (411, 413) that basicities should decrease in the series

La, Ce(III), Pr, Nd, Sm, Eu, Gd, Tb, Dy, Y, Ho, Er, Tm, Yb, Lu, Sc

with thorium and cerium(IV) following in order after scandium. Actinium would be correspondingly more basic than lanthanum, and to obtain elements more basic than actinium, one would have to go to elements of lower positive oxidation numbers and lowered attractions for electrons.

Some further considerations based upon these general ideas have been advanced by von Stackelburg (421), but the essential conclusions remain unchanged.

The relative attractions for electrons and the strengths with which they are held can be approached experimentally by means of ionization potentials and the relative volumes of the free elements and their compounds. Complete ionization-potential data are lacking for the elements in this family, but the

incomplete data (359) listed in table 2 indicate a general increase with increasing atomic number in the rare earth series. This would indicate a corresponding increase in the firmness with which electrons are held. Klemm (217) has postulated an increase in ionization potentials in this series.

In the absence of complete information of this type, further considerations must be based upon atomic and molecular size relationships. Increased attractions for valence electrons must pull these and other electrons closer to the nuclei and produce decreases in size. This is borne out by the atomic volumes of the free elements (218, 311, 354). The values listed in table 2 indicate an increase

TABLE 2
Properties indicative of relative attractions for electrons

ELEM- ENT	ATOMIC NUM- BER	IONIZA- TION POTEN- TIAL (359)	ATOMIC VOLUME (218)	MOLECULAR VOLUMES								
				2R(NO ₃) ₃ ·3M(NO ₃) ₂ ·24H ₂ O					R ₂ (SO ₄) ₃ · 8H ₂ O (411, 412)	R ₂ O ₃ (150)		
				(198)								
				M = Mg	Zn	Mn	Co	Ni				
		<i>e.v.</i>	<i>cc.</i>	<i>cc.</i>	<i>cc.</i>	<i>cc.</i>	<i>cc.</i>	<i>cc.</i>	<i>cc.</i>	<i>cc.</i>	<i>cc.</i>	
Sc....	21										35.53	
Y....	39		20.46						240.8		45.13	
La...	57	5.49	22.43	768.3	763.8	778.6	765.5	759.7		50.28		
Ce...	58	6.91	20.70	764.2	755.5	771.6	757.5	751.5		47.89		
Pr...	59	5.76	20.79	758.0	751.0	769.3	751.0	744.3	253.9	46.65		
Nd...	60	6.31	20.62	761.2	750.0	771.0	748.0	745.3	252.4	46.55		
Sm...	62	6.55	21.70*	742.4	732.8	750.3	739.2	727.7	247.9		48.38	
Eu...	63		29.00						247.3		48.28	
Gd...	64	6.65	19.79	723.0	717.5		720.5	707.0	246.4		47.58	
Tb...	65	6.74	19.11								46.38	
Dy...	66	6.82	18.97						242.8		45.49	
Ho...	67		18.65†						241.1		44.89	
Er...	68		18.29						239.3		44.38	
Tm...	69		18.12								44.11	
Yb...	70	7.06	24.76						235.1		42.5	
Lu...	71		17.96						234.7		42.25	

* Reference 36.

† Reference 35.

from yttrium to lanthanum, followed by a general decrease in the rare earth series from lanthanum to lutecium. Among the few apparent anomalies in the data are the excessively large atomic volumes of europium and ytterbium. These may well be due to the fact that these two elements crystallize in the cubic system, whereas the others are all hexagonal. If one keeps in mind the experimental difficulties which attend the preparation of these elements in the pure condition, he must agree that such data lend excellent support to von Hevesy's conclusions.

A somewhat more exact approach can be made through measurements of the molecular volumes of various compounds. The literature contains many refer-

ences to these measurements of molecular volume (7, 15, 16, 17, 34, 42, 150, 198, 299, 300, 315, 324, 334, 411, 412, 420). In all instances where data are available, increases in molecular volumes in the series scandium–yttrium–lanthanum are noted. Among the rare earth elements, however, while decreases often parallel increases in atomic number, there are many irregularities, and in some instances (17) increases are noted. Such irregularities can be ascribed either to lack of strict isomorphism in the series of compounds compared or to lack of purity in the samples used. The unreliability of data in the older literature may be due to a combination of both factors. Representative data for double nitrates of the type $2R(NO_3)_3 \cdot 3M(NO_3)_2 \cdot 24H_2O$ (198), for hydrated sulfates of the type $R_2(SO_4)_3 \cdot 8H_2O$ (411, 412), and for sesquioxides (150), where in all instances definite isomorphism exists, are included in table 2. Except for slight deviations with the double neodymium nitrates, the expected trends are noted.

The x-ray measurements of Goldschmidt and his coworkers (150) upon the sesquioxides are classic, since the decrease in size relationships in the rare earth series (Goldschmidt's "lanthanide contraction") was first made definitely apparent by this work. The sesquioxides were found to be of three types: A (hexagonal, existent at the highest temperatures), B (pseudotrigonal, existent at intermediate temperatures), and C (regular, existent at the lowest temperatures). By the method used, molecular volumes were found for the A oxides for lanthanum, cerium, praseodymium, and neodymium; for the B oxides for samarium and europium; and for the C oxides for the other rare earth elements from samarium through lutecium, yttrium, and scandium. For any type of oxide, a decrease in molecular volume parallels an increase in atomic number among the rare earth elements, and for the C oxides yttrium gave a value between those for dysprosium and holmium, while scandium was smaller than lutecium. These data thus support conclusions based upon electronic configurations, and decreases in basicity may be expected to parallel decreases in molecular volume. These data and the conclusions based upon them have been discussed by von Hevesy (414), Mark (267), von Stackelburg (421), Bommer (34), and Klemm (217). The reevaluated lattice constants a for the C-type oxides of many of the rare earth elements (34) included in table 3 also indicate the lanthanide contraction.

Of equal importance are the measurements of von Hevesy (411, 412) on the molecular volumes of the octahydrated sulfates. Lanthanum sulfate forms no octahydrate, and octahydrated cerous sulfate is not isomorphous with octahydrated sulfates of the other elements. With the other elements, however, complete isomorphism exists. The lanthanide contraction is again apparent, with yttrium occupying a position between holmium and erbium. The relatively small decreases between samarium and europium and between ytterbium and lutecium can be taken as indicative of small basicity differences in these regions.

Since alterations in the magnitude of molecular volumes are dependent upon alterations in the size of the cations themselves, it is more fundamental to consider the sizes of these cations. Goldschmidt (148, 149) has derived a set of empirical ionic radii from his x-ray data. Corresponding sets of "actual" radii

of the trivalent ions have been calculated by Grimm and Wolff (152) from Goldschmidt's (150) and von Hevesy's (411, 412) data. Both empirical and actual radii are included in table 3, most of the latter values being averages based upon the two sets listed by Grimm and Wolff.

The expected increase in the series scandium-yttrium-lanthanum is followed by a decrease from lanthanum to lutecium, a decrease which becomes so pronounced that the size of the trivalent yttrium ion is reached between dysprosium and holmium. It is apparent that the largest of these cations would have the least tendency to attract electrons or anions and would, therefore, be the most

TABLE 3
Properties related to sizes

ELEMENT	ATOMIC NUM- BER	LATTICE CON- STANT <i>a</i> (34)	RADIUS OF R ⁺⁺⁺		IONIC-POTENTIAL DATA								Δ <i>V</i> / <i>m</i> [*] (393)
			Empiri- cal (148, 149)	Actual (152)	Empirical radii						Actual radii		
					+2		+3		+4		+3		
					φ	√φ	φ	√φ	φ	√φ	φ	√φ	
Sc...	21		0.83	0.68 ₁ *			3.62	1.90			4.40	2.10	0.984
Y....	39		1.06	0.82 ₇			2.83	1.68			3.63	1.91	0.936
La....	57		1.22	1.00 ₄ *			2.46	1.57			2.99	1.73	0.792
Ce....	58		1.18	0.93 ₉ *			2.54	1.59	3.92	1.98	3.20	1.79	0.806
Pr...	59		1.16	0.91 ₆			2.58	1.61	4.00	2.00	3.30	1.82	0.819
Nd...	60	11.05 ₆	1.15	0.90 ₆			2.61	1.62			3.33	1.83	0.833
Sm...	62	10.89 ₃	1.13	0.87 ₂			2.65	1.63			3.44	1.85	0.861
Eu....	63	10.84 ₂	1.13	0.87 ₀	1.71	1.31	2.65	1.63			3.45	1.86	0.875
Gd....	64	10.79 ₇	1.11	0.86 ₂			2.70	1.64			3.48	1.87	0.889
Tb....	65	10.69	1.09	0.84 ₅ *			2.75	1.66	4.49	2.12	3.55	1.88	0.903
Dy...	66	10.62 ₉	1.07	0.83 ₃			2.80	1.67			3.59	1.89	0.917
Ho...	67		1.05	0.82 ₃			2.86	1.69			3.63	1.90	0.931
Er...	68	10.50 ₃	1.04	0.81 ₃			2.88	1.70			3.67	1.91	0.944
Tm...	69	10.45 ₃	1.04	0.81 ₂ *			2.88	1.70			3.69	1.92	0.958
Yb....	70	10.40 ₃	1.00	0.79 ₆	1.79	1.34	3.00	1.73			3.80	1.95	0.972
Lu....	71	10.37 ₃	0.99	0.78 ₇			3.03	1.74			3.81	1.95	0.986

* Based upon oxides only.

basic. A decrease in basicity should then parallel a decrease in cation radius, lanthanum being the most basic, scandium the least, and yttrium falling between dysprosium and holmium.

Perhaps the most significant is the prediction of a decrease in basicity with increase in atomic number among the rare earth elements. This is but one of the effects produced by the lanthanide contraction upon the chemical properties of both the rare earth elements and the elements which immediately follow them in the periodic classification. These effects have been discussed comprehensively by a number of authors (15, 80, 133, 151, 215, 217, 267, 411, 413, 421, 452).

The relative sizes of these materials in states of oxidation other than positive three have been only incompletely evaluated. Goldschmidt (150) indicated the

molecular volumes of the dioxides of cerium, praseodymium, and terbium to be 47.98, 46.65, and 39.23 cc., respectively. The corresponding radii for the tetravalent cerium, praseodymium, and terbium ions are 1.02, 1.00, and 0.89 Å. (148, 161), respectively. Empirical radii for the divalent europium and ytterbium ions have been given as 1.17 Å. (330) and 1.06 Å. (372), respectively. From size considerations, one would predict these very similar elements to be less basic in the tetravalent state and more basic in the divalent state than in the trivalent state. Inasmuch as the radius of the europous ion approaches that of the strontium ion very closely (219, 330), while that for the ytterbous ion is approximately the same as that of the divalent calcium ion (372), the basicities of divalent europium and ytterbium should approach those of the corresponding alkaline earth elements. The instabilities of these lower oxidation states in aqueous solution have precluded experimental verification of this prediction.

While considerations based upon ionic sizes alone serve well for comparisons among elements in a given and constant state of oxidation, they are inadequate in comparisons involving two or more states of oxidation. Since the relative attractions for electrons are also influenced by the magnitudes of the cationic charges, charge effects must also be considered. An interesting quantity, termed the ionic potential, has been suggested by Cartledge (77, 78) to summarize the combined effects of cation charge and radius. This is defined as

$$\text{Ionic potential} = \phi = \frac{\text{cation charge}}{\text{cation radius}}$$

and Cartledge has stated that a hydroxide will be basic, amphoteric, or acidic when the square root of the ionic potential becomes less than 2.2, between 2.2 and 3.2, and greater than 3.2, respectively. It follows that the more basic a hydroxide is, the smaller the ionic potential for the cation in question. More recently, this idea has been extended by Sun (392), who reported ionic potentials of less than 6 for basic hydroxides and more than 6 for acidic materials; the smaller the value the greater the basicity, and *vice versa*. Ionic-potential values calculated from both empirical and actual ionic radii are listed in table 3. Comparatively high basicities are indicated for the trivalent materials, somewhat larger for the divalent, and somewhat smaller for the tetravalent. Indicated decreases in basicities in a given oxidation state parallel those given by ionic radii alone.

Sun and Li (393) have proposed as a measure of basicity the magnitude of the relation AV/n^3 , where A represents the atomic number, V the valence or oxidation number, and n the principal quantum number of the highest quantum level present in the neutral atom. According to these authors, a hydroxide will be basic, amphoteric, or acidic when values of this expression are respectively less than 1.44, around 1.44, or greater than 1.44, with basicity increasing as the magnitude decreases. Sun and Li's values for the positive three state of oxidation are listed in table 3. An increase in basicity in the series scandium-yttrium-lanthanum is again indicated, followed by a decrease from lanthanum to lutetium. Yttrium would be placed between holmium and erbium, but scandium

would have to precede lutecium. Except for this anomaly, basicity trends are accurately indicated, and the values given would give the elements in Group IIIA larger basicities than those in Group IIIB but smaller basicities than the elements in Group IIA.

In summary, it can be said that considerations based upon the relative binding strengths of the valence electrons in the neutral atoms or upon attractions of electrons exhibited by the derived cations predict basicity decreases in the series

La, Ce(III), Pr, Nd, Sm, Eu, Gd, Tb, Dy, Y, Ho, Er, Tm, Yb, Lu, Sc, Ce (IV)

the position of yttrium being either as indicated or between holmium and erbium. While the tetravalent materials would be expected to be less basic than the tri-valent, they would possess only very slight acidic properties. It is of interest that Brauner reported in an early summary (53) such a stepwise decrease in basicity from lanthanum to scandium.

B. EXPERIMENTAL APPROACHES

1. *Order of precipitation by soluble alkaline materials*

The classical method of establishing relative basicities involves a determination of the order in which hydrous oxides or hydroxides precipitate from mixed salt solutions upon the gradual addition of some soluble alkaline material such as ammonia or sodium hydroxide. Since such precipitation involves the consumption of hydroxyl ions, those cations binding hydroxyl ions most strongly into the most insoluble compounds will precipitate first and be followed in order by materials with less and less affinity for the hydroxyl group. This amounts to precipitation in the order of increasing basicities, the most basic material precipitating last (33, 180, 241, 250, 285, 379).

While such precipitation should parallel the relative decrease in attraction for electrons, there are certain complicating factors. Thus, if basicities are to be compared exactly in this fashion, it must be assumed that the dissolved portions of the hydrous hydroxides are very largely dissociated (250) or at least that the hydroxides of all the elements in this family are dissociated to approximately the same extent. Since such assumptions are not unreasonable in view of the slight solubilities and relatively high basicities of the hydrous precipitates and the striking similarities existent among the compounds, such orders of precipitation appear to be reasonable, even though not exact, measures of variations in basicity (155).

Experimentally, such measurements have usually been made upon natural mixtures of the various elements. As a consequence, the results may very well be influenced by the varying ratios of the elements present (283). Unless localized excesses of the added alkali be avoided, say by excessive dilution, more abundant members may be precipitated out of order in the basicity arrangement (33), and whether these go back into solution and reprecipitate in their true places is then a function of the time of digestion and the total quantity of ma-

terial present. The apparently anomalous position ascribed to yttrium may be due to concentration effects (398).

Innumerable general references to orders of precipitation have appeared. Because of the lack of recognition of individual elements by the early workers and the inadequacy of the analytical methods often employed, much of this information is certainly of doubtful accuracy. Excellent summaries of early work have been given by Urbain (401), who listed the elements in order of decreasing basicity as

La, Di, Ce(III), Y, Gd, Sm, Tb, Ho, Er, Tm, Yb, Sc

and particularly by Böhm (33), who gave the order as

La, Pr, Nd, Gd, Sm, Y, Tb, Ho, Er, Tm, Yb, Sc, Ce(IV)

and pointed out that while the order lanthanum, praseodymium, neodymium appeared definite, the remainder of the series was in doubt. These series were based upon such observations as the following: the precipitation of didymium before lanthanum (29); the precipitation of samarium before gadolinium (16, 24, 109); precipitation of the erbium earths, terbium, and yttrium in that order (170); the grouping of lanthanum, didymium, samarium, yttrium, and terbium in the more basic fractions with erbium, holmium, thulium, ytterbium, scandium, and cerium in the less basic ones upon ammonia precipitation (231); increase of basicity in the series ytterbium, erbium, holmium, terbium as shown by ammonia precipitation (261); precipitation of didymium in preference to lanthanum with magnesium oxide (310); precipitation of samarium before didymium with ammonia (90); precipitation of samarium, didymium, and lanthanum in that order with ammonia (94); concentration of yttrium in the more basic fractions in ammonia separations (107, 305); and precipitation in order of the colorless yttrium earths, terbium, erbium, samarium, neodymium, and praseodymium by ammonia (309).

This same general order as determined by alkali precipitation is also given in later papers. Thus, Meyer (278) listed in order of decreasing basicity:

La, Pr, Nd, Ce(III), Y, Sm, Gd, Tb, Ho, Er, Tm, Yb, Sc, Ce(IV)

Levy (241) gave

La, Ce(III), Pr, Nd, Y, Eu, Gd, Sm, Tb, Dy, Ho, Er, Tm, Yb, Lu, Sc, Ce(IV)

and Spencer (379, 384) listed

La, Ce(III), Pr, Nd, Gd, Sm, Y, Tb, Dy, Ho, Er, Tm, Yb, Sc, Ce(IV), Th

Peculiar to most of these arrangements are the listing of gadolinium as a more basic element than samarium and the placing of yttrium with the relatively strongly basic cerium earths. The first of these is ascribable to early observations that ammonia precipitated samarium before gadolinium from natural mixtures (16, 24, 109). The comparatively high basicity thus assigned to gado-

linium led Meyer and Hauser (285) to postulate a discontinuity in basicity among the rare earth elements, an increase at gadolinium following a regular decrease among the cerium earths. Two parallel series of decreasing basicities thus resulted:

La, Ce(III), Pr, Nd, Sm

and

Y, Gd, Tb, Er, Yb, Sc, Ce(IV)

This arrangement was cited by Renz (353) as indicative of periodicity within the rare earth group and is supported by Brauner's work on the hydrolysis of sulfates (55, 59, 60).

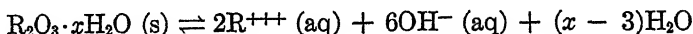
Establishment of the relative basicities of samarium and gadolinium by ammonia precipitation has recently been clarified by Günther, Kotowski, and Lehl (155). Fractional precipitation of synthetic mixtures containing equivalent amounts of the two elements, followed by quantitative analysis of each fraction by an x-ray method, indicated samarium to be definitely the more basic, although the differences found were small in comparison with those existing in other regions of comparable difference in atomic number. The variance between these results and those of earlier workers (16, 24, 109) was ascribed to alteration in the normal order of precipitation when large amounts of gadolinium are separated from small amounts of samarium in the presence of other materials and to lack of sensitivity in early methods of analysis.

The position of yttrium with the cerium earths is anomalous in the light of considerations based upon size and charge-size relationships which would predict its basicity to lie in the neighborhood of that of holmium. Yet the correctness of this position as based upon natural mixtures is borne out by the widespread use of basic precipitation as a method of removing yttrium from holmium and erbium. Inasmuch as yttrium is by far the most abundant element in the yttrium subgroup, concentration effects may well determine its position. This is implicit in the data of Trombe (398), which indicate that the precipitation pH of hydrous yttrium hydroxide falls markedly as the concentration increases. Further investigations upon synthetic mixtures of yttrium with other earths should settle this point.

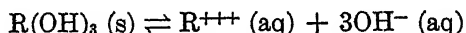
2. Precipitation and dissolution of hydrous oxides and hydroxides

The natures of the gelatinous precipitates thrown down by the action of soluble alkalis upon aqueous rare earth salt solutions or produced by the action of water upon the corresponding oxides have been only incompletely ascertained (427). Thus, while dehydration experiments show that lanthanum (186), praseodymium (428), and neodymium (428) yield hydrous hydroxides, yttrium and samarium (428) hydrous oxides, and scandium (428) a hydrous monohydrate, corresponding data are not available for the remaining elements. While basic salts undoubtedly separate first in many alkali precipitations (343, 376), at least some of the other elements appear to form hydrous hydroxides under these conditions (144).

Regardless of the nature of the hydrous material precipitated, its precipitation involves the consumption of hydroxyl ions and is, therefore, related to the basicity of the element in question. Correspondingly, the water solubility of such a material is in turn a measure of the release of hydroxyl ions and involves the basicity of the element in question. These facts are apparent in the equilibria (297)



and



which are involved in both the precipitation and the dissolution processes. It is apparent also that dissolution in acidic materials also measures basicity, since it involves the removal of the equilibrium amounts of hydroxyl ions. As methods of establishing relative basicities through measurements of the relative displacements of these equilibria, one can cite determinations of solubilities, solubility-product constants, and precipitation pH values.

(a) Miscellaneous measurements of solubilities and solubility-product constants

Although the literature contains many such qualitative statements as that the sesquioxide of praseodymium is less soluble in water than that of lanthanum (307), few quantitative measurements of water solubilities have been made. Busch (74), by digesting oxides with water at 29°C., filtering, and titrating the liberated hydroxyl ions with dilute acid, obtained the following solubilities (expressed as gram-moles of the sesquioxide dissolved per liter ($\times 10^6$)): lanthanum, 12.3; praseodymium, 0.61; neodymium, 5.75; yttrium, 8.0; erbium, 12.8. While he believed that solubilities should decrease with decreasing basicities, Busch pointed out that solubilities so determined were functions of the temperature and time of ignition of the oxide as well as its previous history. The order of decreasing basicity (erbium, lanthanum, yttrium, neodymium, praseodymium) derivable from these data cannot, therefore, be significant.

By digesting lanthanum oxide with water at 25°C. for 10 days, evaporating the clear liquor, adding excess acid, and back-titrating with alkali, Kolthoff and Elmquist (222) found this oxide to dissolve to the extent of 0.73 mg. (2.2×10^{-6} g.-mole) per liter. This corresponds to a solubility-product constant for the hydroxide of 0.91×10^{-21} . Similar investigations have not been carried out for the other materials.

Most observations on the relative solubilities of the oxides or hydrous precipitates in acidic solutions have been only qualitative, although Brauner (45) early pointed out that the solubilities in aqueous ammonium nitrate solution of Di_2O_3 , Di_2O_3 , and La_2O_3 were in the ratio of 1 to 10 to 29. More recently Prandtl and coworkers (335, 342, 343, 344, 345, 346) have measured the solubilities of a number of hydroxides in ammonium salt solutions in the presence and absence of cations forming ammonia complexes. Their data indicate solubility increases

(and basicity increases) in the order yttrium, samarium, neodymium, praseodymium, lanthanum.

Prändtl's experimental approach has been extended by Endres (133) to an interesting study of the relative basicities of the trivalent rare earth elements and yttrium in terms of the solubility-product constants for their hydrous hydroxides. Through application of the usual solubility-product treatment to the reactions between hydroxyl ions and the cations derived from two elements, R and R', followed by the assumption that if both precipitations were made in ammonia-ammonium nitrate-cadmium nitrate buffers the equilibrium hydroxyl-ion concentrations would be essentially the same, Endres derived the relation

$$\frac{[\text{R}^{+++}]}{[\text{R}'^{+++}]} = \frac{K}{K'}$$

in which K and K' represent the solubility-product constants of $\text{R}(\text{OH})_3$ and $\text{R}'(\text{OH})_3$, respectively.

Making the further assumption that the ratio of the solubility-product constants is the same as the ratio of the dissociation constants for the hydroxides, Endres then pointed out that the ratio of the analytically determined concentrations of the two ions in question in two buffers of the same pH would represent not only the ratio of the two solubility products but the ratio of the basicities as well. Thus, to use his example, if

$$[\text{Pr}^{+++}]/[\text{Nd}^{+++}] = 1.7$$

praseodymium hydroxide would be 1.7 times as basic as neodymium hydroxide. That the ratio of solubility-product constants also represents the ratio of basicities can be true only if these hydroxides are all of about the same solubility and are largely dissociated in solution (250), conditions which, although closely approached, are not exactly attained. Herein lies the weakness of Endres's method.

Endres determined the concentrations of lanthanum, praseodymium, neodymium, samarium, gadolinium, yttrium, and dysprosium ions existing at 100°C. in ammonia-ammonium nitrate-cadmium nitrate buffers of varying cadmium content. The solubilities of materials less basic than dysprosium were so small that measurements with such materials could not be made. From these data basicities of the various hydroxides relative to yttrium were calculated in accordance with the above considerations. These results are summarized in table 4 and represent the numerical values often quoted by other authors (178, 179, 374). The comparatively large difference between lanthanum and praseodymium and the small differences between praseodymium and neodymium and between samarium and gadolinium are in accord with the results of basicity separation procedures. The order of decreasing basicities, except possibly for dysprosium, is that indicated by theoretical considerations.

Endres (133) recognized the dependence of basicity upon ionic radii and pointed out parallel variations in relative basicities and ionic radii. This interdependence is strikingly shown in figure 1, where the logarithms of the relative basicities (solubility-product ratios) and the ratios of the calculated ionic radii

(152) to the radius of the yttrium ion are both plotted against atomic numbers (297).

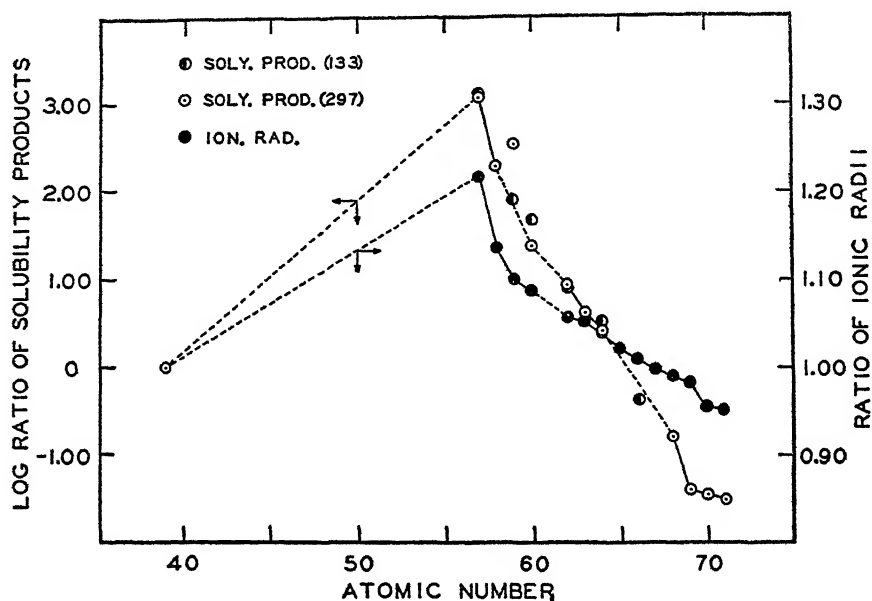


FIG. 1. Relative basicities and ionic radii

TABLE 4
Relative basicities of rare earth hydroxides

RATIO	RATIO OF SOLUBILITY-PRODUCT CONSTANTS		RATIO OF IONIC RADII (152)
	Endres (133)	Moeller and Kremers (297)	
La:Y.....	1300	1235	1.214
Ce(III):Y.....		185	1.135
Pr:Y.....	80	333	1.100
Nd:Y.....	47	23.5	1.089
Sm:Y.....	8	8.4	1.055
Eu:Y.....		4.2	1.051
Gd:Y.....	3.4	2.6	1.041
Tb:Y.....			1.021
Dy:Y.....	0.5		1.010
Y:Y.....	1.0	1.0	1.000
Ho:Y.....			0.999
Er:Y.....		0.16	0.990
Tm:Y.....		0.041	0.983
Yb:Y.....		0.036	0.955
Lu:Y.....		0.031	0.952

On the basis of an extension of these ideas to elements the relative basicities of which could not be measured by the method used, Endres (133) classified

the rare earths into the neodymium (neodymium to No. 61), gadolinium (samarium to gadolinium), erbium (terbium to thulium), and ytterbium (ytterbium to lutecium) earths. Klemm (215) has seriously objected to this classification on the ground that it represents too much of an extension beyond experimental data.

(b) Measurements based upon data from electrometric titrations

Data obtained when the changes in pH during the titration of metal salt solutions with alkalis are measured electrometrically by means of hydrogen, oxygen, glass, or other electrodes not only permit evaluation of solubility-product constants but indicate precipitation pH values as well. Britton (66) suggested that an arrangement of the metal ions in the order of the pH values at the incidence of precipitation of their hydroxides may also represent a basicity arrangement but stated that it may not. Although precipitation pH is closely related to solubility-product constant, it has been suggested recently that inasmuch as this pH is a measure of the hydroxyl-ion concentration or activity in an equilibrium of the type discussed in the preceding section, it must also be a measure of the extent to which the hydroxyl ion is lost by the hydrous oxide or hydroxide, or in other words the basicity (297).

Precipitation pH data have been reported by a number of investigators. Thus, Hildebrand (167) found praseodymium and neodymium chloride solutions to yield precipitates in pH ranges close to 7, whereas the corresponding nitrate solutions gave precipitates at pH values below 4, a difference which has been ascribed to catalytic reduction of the nitrate ion at the hydrogen electrode used (64).

The pH of saturated lanthanum hydroxide solution was found electrometrically to be 6.55 (409), and the equilibrium pH of lanthanum hydroxide in contact with acetic acid was determined by a similar method to be 8.63 (410). The first of these values is undoubtedly too low, in the light of other evidence.

Britton (64, 65), working with a hydrogen electrode in solutions approximately 0.01 *M* in rare earth ions, investigated the effects of sodium hydroxide upon the nitrates and chlorides of lanthanum, cerium(III), praseodymium, neodymium, samarium, and yttrium at temperatures of 17° to 18°C. While hydrous lanthanum hydroxide precipitated at a pH of 8.35, all the others came down in a comparatively narrow pH range between 7.14 (cerium) and 6.78 (yttrium). The numerical values are summarized in table 5. In spite of the small differences, an observable decrease was noted in the series lanthanum, cerium, praseodymium, neodymium, samarium, yttrium, and basicities were stated to decrease in this order. Precipitation of thorium material at a pH of 3.5 indicated this tetravalent element to be much less basic than the trivalent rare earth elements and yttrium. In his scale of relative basicities, Britton (66) places the rare earth elements and yttrium below magnesium and divalent manganese and above cobalt and nickel, with the statement that only lanthanum and cerous hydroxides can be regarded as being moderately strong bases.

In a series of electrometric titrations at 18°C., Sadolin (361) investigated the

precipitation of hydrous lanthanum hydroxide with sodium hydroxide. Although the pH of precipitation incidence is not exactly indicated in these data, a reasonable constancy in pH during titration is shown in the neighborhood of 8.4.

TABLE 5
Precipitation pH values for hydroxides of trivalent elements

ELEMENT	RADIUS OF R^{+++} Å	pH						
		At precipitation incidence				At $OH^-/R^{+++} = 0.4$ (297)		
		NO_3^-	Cl^-	$C_2H_3O_2^-$	SO_4^{--}	NO_3^-	$C_2H_3O_2^-$	SO_4^{--}
La.	1.00 ₄	8.35 (64) 8.71 (327) 7.82 (297)	8.03 (44)	7.93 (297)	7.61 (44) 7.41 (297)	8.23	8.13	7.78
Ce.	0.93 ₉	8.1 (327) 7.60 (297)	7.41 (64)	7.77 (297)	7.07 (44) 7.35 (297)	7.76	7.99	7.56
Pr.	0.91 ₆	7.35 (297)	7.05 (64)	7.66 (297)	6.98 (44) 7.17 (297)	7.67	7.96	7.50
Nd ...	0.90 ₆	7.00 (64) 7.31 (297)	7.02 (64) 7.40 (44)	7.59 (297)	6.73 (44) 6.95 (297)	7.40	7.65	7.23
Sm.	0.87 ₂	6.92 (297)	6.83 (64)	7.40 (297)	6.70 (297)	7.08	7.48	6.93
Eu.	0.87 ₆	6.82 (297)		7.18 (297)	6.68 (297)	6.90	7.37	6.82
Gd.	0.86 ₂	6.83 (297)		7.10 (297)	6.75 (297)	6.94	7.31	6.95
Y.	0.82 ₇	7.39 (327) 6.95 (297)	6.78 (64)	6.83 (297)	6.83 (297)	6.90	7.15	6.90
Er.	0.81 ₈	6.76 (297)		6.59 (297)	6.50 (297)	6.84	6.93	6.58
Tm.	0.81 ₂	6.40 (297)		6.53 (297)	6.21 (297)	6.70	6.77	6.38
Yb.	0.79 ₆	6.30 (297)		6.50 (297)	6.16 (44) 6.18 (297)	6.65	6.73	6.32
Lu.	0.78 ₇	6.30 (297)		6.46 (297)	6.18 (297)	6.63	6.73	6.32
Sc.	0.68 ₁			6.1 (389)				

Sterba-Böhm and Melichar (389) found the hydrous scandium compound to precipitate at a pH of 6.1 when a 0.01 *M* acetate solution was titrated with potassium hydroxide.

Using a glass electrode and working with solutions stirred with nitrogen to avoid the effects of absorption of carbon dioxide, Bowles and Partridge (44)

followed titrations of approximately 0.01 *M* solutions of lanthanum, cerous, ceric, praseodymium, neodymium, ytterbium, and thorium sulfates and lanthanum and neodymium chlorides with sodium hydroxide at 25°C. While the trivalent sulfates yielded precipitates at somewhat lower pH values than the chlorides, all values lay in the neighborhood of pH 7, with a decrease in pH in the series lanthanum, cerium(III), praseodymium, neodymium, ytterbium, thorium, cerium(IV). Ceric and thorium sulfates yielded precipitates at pH values of 2.65 and 3.91, respectively, indicating much lower basicities for these tetravalent materials.

In a similar study, Oka (327) employed a glass electrode to follow the effects of sodium hydroxide upon approximately 0.005 *M* solutions of lanthanum, cerous, and yttrium nitrates at 25°C. Although the pH values reported for precipitation incidence are somewhat higher than those given by others (table 5), the order of decreasing pH is the same.

In a recent publication (297), similar measurements at 25°C. upon nitrate, sulfate, and acetate solutions approximately 0.1 *M* in the trivalent ions of lanthanum, cerium, praseodymium, neodymium, samarium, europium, gadolinium, erbium, thulium, ytterbium, lutecium, and yttrium have been reported. For a given rare earth element, precipitation pH values increased in the series sulfate, nitrate, acetate, the somewhat higher values for the acetates being ascribed to the increased tendency of the acetate ion to form coördination complexes with the rare earth ions. The lower values for the sulfates were attributed to the flocculating action of this divalent anion upon the intermediate colloidal sols. While in no instance were the differences in precipitation pH between two adjacent rare earth elements particularly large, such differences were usually significant, and steady decreases in precipitation pH paralleled decreases in the radii of the trivalent ions, yttrium occupying its expected position in the vicinity of gadolinium and erbium.

Recognizing the difficulty of noting accurately the initial appearance of precipitates, these authors (297) also reported pH values at a mole ratio of added hydroxyl ion to rare earth ion initially present of 0.4. At this mole ratio, precipitation was regarded as proceeding uniformly in all instances, and the results were then comparable. The same trends were noted, but in a more clearly defined manner.

A somewhat different approach to the pH values corresponding to the incidence of precipitation has been reported by Trombe (398). The slow introduction of gaseous ammonia into comparatively large volumes of rare earth nitrate solutions was shown to give a general rise in pH during the formation of colloidal sols. With the continued introduction of ammonia, flocculation and an abrupt drop in pH were then noted, the minimum pH so attained being termed the pH at the threshold of precipitation. Values found for the cerium earths were in general 0.3 to 0.6 unit higher than those reported by Britton (64) at the same concentrations and were markedly dependent upon the concentrations of the salt solutions used, decreases being noted in all instances with increasing concentration. Over a concentration range of 0.005 to 0.5 *M*, the pH decreases ob-

TABLE 6
Solubility-product constants and solubilities of trivalent hydrous hydroxides

ELEMENT	TEMPERATURE	SOLUBILITY- PRODUCT CONSTANT K	WATER SOLUBILITY S $\times 10^3$	REMARKS	REFERENCE
	°C.		<i>g.-moles per liter</i>		
La.....	18	1.2×10^{-19}		Titration, fresh	(361)
	18	1.1×10^{-21}		Titration, aged	(361)
	25	0.91×10^{-21}		Digestion, aged	(222)
	25	4.3×10^{-19}	9.2	Titration, fresh	(327)
	25	1.0×10^{-19}	7.8	Titration, fresh	(297)
	25	1×10^{-20}		Estimation	(240)
Ce.....	25	0.8×10^{-20}	4.1	Titration, fresh	(327)
	25	1.5×10^{-20}	4.8	Titration, fresh	(297)
Pr.....	25	2.7×10^{-20}	5.4	Titration, fresh	(297)
Nd.....	25	1.9×10^{-21}	2.7	Titration, fresh	(297)
Sm.....	25	6.8×10^{-22}	2.0	Titration, fresh	(297)
Eu.....	25	3.4×10^{-22}	1.4	Titration, fresh	(297)
Gd.....	25	2.1×10^{-22}	1.4	Titration, fresh	(297)
	25	5.2×10^{-22}	1.9	Titration, fresh	(327)
	25	8.1×10^{-23}	1.2	Titration, fresh	(297)
Y.....	25	1×10^{-24}		Estimation	(240)
	25	1.3×10^{-23}	0.8	Titration, fresh	(297)
Tm.....	25	3.3×10^{-24}	0.6	Titration, fresh	(297)
Yb.....	25	2.9×10^{-24}	0.5	Titration, fresh	(297)
Lu.....	25	2.5×10^{-24}	0.5	Titration, fresh	(297)
	25	1×10^{-26}		Estimation	(240)
Sc.....	25	1×10^{-23}		Estimation	(240)

served were: lanthanum, 1.44; praseodymium, 0.85; neodymium, 0.65; samarium, 0.39; gadolinium, 0.80; dysprosium, 0.75; ytterbium, 1.25; yttrium, 2.14.

These uneven variations in precipitation pH with concentration were found to yield different orders of precipitation at different concentrations. Thus the order of decreasing pH in 0.005 *M* solutions was

La, Y, Pr, Nd, Sm, Gd, Yb, Dy

while in 0.5 *M* solutions it was

The alteration observed with yttrium was far larger than that with any other element, and the author advanced the opinion that this alteration is responsible for the varying position occupied by yttrium in basicity series determined in a variety of fashions. Further suggestions as to the optimum concentrations for effecting separations by basic precipitation were made.

A summary of the precipitation pH data available is given in table 5, together with the corresponding references. For comparison, the calculated ionic radii of Grimm and Wolff (152) are also included. Agreement among the values from various sources is excellent, considering the variety of temperatures and concentrations employed. The parallel decreases in precipitation pH values and ionic radii are again indicative of the interdependency of size and basicity.

Solubility-product constants have been calculated from titration data by Sadelin (361), Oka (327, 328), and Moeller and Kremers (297), upon the assumption of the ultimate precipitation of hydrous hydroxides in all instances. That basic salts actually form in such reactions is well known (343), but the method is still useful particularly for comparisons of the freshly precipitated materials. Latimer (240) has also estimated solubility-product constants for the hydroxides of lanthanum, yttrium, lutecium, and scandium from available electrochemical data. A summary of such solubility-product constants (K) and corresponding water solubilities (S) as are available is given in table 6, together with appropriate references. The values listed from the paper by Moeller and Kremers (297) are averages for nitrate, sulfate, and acetate solutions. Decreases in solubility products and solubilities parallel decreases in ionic radii and may be taken as indicating corresponding decreases in basicity (297).

Relative basicities as calculated from actual solubility-product constants (297) in accordance with the method of Endres (133) are listed in table 4 and plotted in figure 1. The agreement between these values and those given by Endres is excellent, except for praseodymium (297), and the parallel between ionic radii and basicities is readily apparent from the graph.

(c) Summary

Measurements based upon physicochemical investigations of the equilibria between hydroxyl and rare earth ions in aqueous solutions indicate a decrease in basicity corresponding to a decrease in cation size, yttrium occupying its expected position somewhere between gadolinium and erbium and scandium giving the least basic trivalent hydroxide. The basicity of lanthanum hydroxide so determined is considerably larger than the values for the other materials, and tetravalent cerium yields a hydroxide which is less basic than any of the trivalent materials. Basicity differences in the regions of samarium to gadolinium and thulium to lutecium are comparatively small.

3. Hydrolysis studies

The literature is replete with qualitative references to the slight hydrolysis in aqueous solution of salts of yttrium and the trivalent rare earth elements with weakly basic anions. Thus, solutions of the bromides of cerium, neodymium,

tral to methyl orange (249); salts of strong acids were found not to hydrolyze (43); lanthanum bromide was only slightly hydrolyzed (383); dysprosium chloride solution was found to be nearly neutral to litmus (134); lanthanum sulfate solutions were "neutral" (58, 205); lanthanum and cerous acetates were not strongly hydrolyzed (248); and lanthanum chloride was found to be less hydrolyzed than the chloride of any other trivalent metal (203, 204). Scandium salts, however, appear to be more highly hydrolyzed (28, 282, 388, 389), as is to be expected from the somewhat smaller size of the scandium ion. In the presence of fairly strongly basic anions such as the azide or nitrite, hydrolysis is more extensive (2, 98, 172) and may become nearly complete at elevated temperatures (2, 98, 225).

The only non-trivalent ion stable in aqueous solution is the ceric ion. The combined effects of an increased cation charge and a decreased size render ceric salts much more highly hydrolyzed in aqueous solution than those of any of the trivalent ions. As examples of this increased hydrolysis, one may cite the strongly acidic reactions of ceric salt solutions (257, 288), the lack of existence of normal ceric salts of weak acids (257), the ready precipitation of tetravalent cerium with azides (2) or sodium acetate (289), the tendency for basic salts to form from solution (139, 158, 164, 284, 378), the formation of colloidal ceric oxide by high temperature (197) or dialytic (26, 27) hydrolysis, and the many methods of separating cerium from the other materials by hydrolysis after oxidation (see Part III).

(a) Chemical studies

A comparison of the extent of hydrolysis of rare earth sulfate solutions in terms of the effects of liberated acid upon the interaction of iodate and iodide in the equation



has been made by Katz and James (211). Approximately 0.08 *N* rare earth sulfate solutions were treated with potassium iodide and iodate solutions and steam distilled, the liberated iodine in the distillates being titrated with standard sodium thiosulfate. The quantities of iodine liberated increased in the series lanthanum, cerium(III), neodymium, samarium, europium, gadolinium, erbium, and ytterbium, corresponding to increased displacements of the above equilibrium. This series then represents a series of increasing hydrolysis or decreasing basicity.

The decrease in basicity so found paralleled an increase in atomic weights, but there was no sharp division of the rare earth elements into two series. Although only slight decreases were noted in the series samarium-europium-gadolinium, gadolinium was definitely the least basic, and the authors were of the opinion that in separations involving these elements gadolinium should concentrate in the least basic fractions rather than in the most basic ones as previously noted (16, 109).

In an attempt to reconcile the anomalously high basicity of yttrium as indi-

cated by basic precipitation, Brinton and James (63) determined the extents to which a number of carbonates hydrolyzed. Aqueous 0.1 *N* sulfate solutions were treated with sodium carbonate and boiled for varying periods of time, the evolved carbon dioxide being absorbed and determined. The degree of hydrolysis was then calculated from the ratio of the carbon dioxide evolved to the total carbon dioxide initially in the system. While lanthanum and cerous carbonates hydrolyzed completely in the first half hour, hydrolysis increased slowly with the others as boiling was continued. Lanthanum and cerium being omitted, the observed order of increasing hydrolysis and decreasing basicity was praseodymium, neodymium, samarium, europium, gadolinium, terbium, dysprosium, yttrium, thulium, ytterbium. Yttrium fell between dysprosium and thulium, and the authors believed that it would have fallen between erbium and holmium, had these materials been investigated. Further investigation of the hydrolysis of rare earth carbonates by Preiss and Dussik (347) indicated the rare earths to stand between aluminum and the alkaline earths in basicity.

The relative basicities of a number of these elements have been measured by Brauner and a coworker (55, 59, 60) through determinations of the effects of solutions of rare earth sulfates upon the hydrolysis of methyl acetate and the inversion of sucrose, both reactions being acid-catalyzed. In a series of preliminary measurements, Brauner (55) found that the rare earths group themselves into two parallel series of decreasing basicities: namely, lanthanum, (cerium), praseodymium, neodymium, samarium and gadolinium, terbium, erbium (ytterbium). More careful measurements confirmed the existence of these two series, with gadolinium beginning the second (59, 60). From the hydrolysis of methyl acetate, the percentages of hydrolysis of the rare earth sulfates in *N*/32 solution were calculated as: lanthanum, 0.48; cerium, 0.59; praseodymium, 1.59; neodymium, 1.80; samarium, 6.14; gadolinium, 0.80; ytterbium, 2.57.

The existence of a discontinuity at gadolinium represents somewhat of an anomaly in determinations of basicity. Brauner was concerned primarily with the periodic classification of the rare earth elements and felt that gadolinium introduced a new series of elements. While breaks in properties often occur at gadolinium (215, 216, 217), other methods of determining basicities do not indicate such a break.

Many years ago, Ley (248) found the inversion of sucrose and the hydrolysis of methyl acetate to be only slightly catalyzed by lanthanum chloride and calculated that lanthanum chloride was only 0.4 per cent hydrolyzed. He noted that ceric nitrate was considerably hydrolyzed and mentioned that cerous chloride was hydrolyzed to the extent of about 0.5 per cent. The other materials were not investigated.

The sodium nitrite separation procedure of Holden and James (172) involves fractional precipitation of the elements as a result of the hydrolysis of nitrite in boiling solutions. Sherwood (373, 374) applied this general procedure to a determination of the relative basicities of the rare earth elements through fractionation of a cerium-free concentrate and determination of the order of precipitation by measurements of absorption spectra and magnetic susceptibilities.

A general decrease in basicity with increasing atomic numbers was noted, although in the series samarium-europium-gadolinium the decrease was very small.

Hughes and Hopkins (185) extended this procedure to a determination of the relative basicities of yttrium and illinium, the position of yttrium in the basicity fractionation being established by measurements of arc spectra and that of illinium by magneto-optic measurements. Both elements were found to be between neodymium and samarium in basicity, with illinium being more basic than yttrium. Nitrite precipitation thus indicates decreasing basicity in the series

La, Pr, Nd, Il, Y, Sm, Eu, Gd, Tb, Dy, Ho, Er, Tm, Yb, Lu

the position of yttrium being about that obtained by other methods upon natural mixtures.

A somewhat similar determination based upon the hydrolysis of azides has been carried out by Ant-Wuorinen (2). Treatment of a mixed chloride or nitrate solution with sodium azide followed by heating precipitated the materials as basic salts in the order

Ce(IV), Sc, Yb, Tm, Er, Ho, Dy, Gd, Tb, Eu, Sm, Y, Ce(III), Nd, Pr, La

as determined by spectroscopic analysis. This order of increasing basicities agrees fairly well with other orders.

Vesterberg (407, 408) has shown that the strengths of slightly soluble inorganic bases can be measured by determining the extents of hydrolysis of the corresponding acetate solutions through extraction of the liberated acetic acid and titration, account being taken of the partition coefficient of acetic acid between water and ether. By this method, lanthanum acetate was found to be 0.315 per cent hydrolyzed in *N*/5 solution and 0.286 per cent in *N*/10, with an average of 0.30 per cent, at 18°C. (406, 407, 408). Extension of this procedure to scandium and yttrium acetate solutions (389) has indicated the respective extent of hydrolysis in *N*/10 solutions to be 11.40 per cent and 0.71 per cent. A significant increase in basicity in the series scandium-yttrium-lanthanum is indicated.

(b) Physicochemical studies

(1) Conductivities of aqueous salt solutions

Deviations in the conductivities of aqueous salt solutions from values characteristic of the particular salt type may often be ascribed to hydrolysis. Furthermore, the degree of hydrolysis can be calculated from such data.

Numerous conductivity studies upon solutions of salts of scandium, yttrium, and the rare earth elements have been reported (28, 55, 59, 60, 81, 200, 203, 206, 207, 220, 222, 248, 258, 281, 282, 307, 326, 355, 360). While some of the data are doubtless inaccurate because of the use of relatively impure materials, most of the results are in sufficiently good agreement to permit comparisons.

The conductivity data are such that most authors agree that yttrium and rare earth salts of the types RCl_3 , $R(NO_3)_3$, and $R_2(SO_4)_3$ cannot be extensively hydrolyzed even in highly dilute solutions (28, 200, 242, 360). Scandium compounds, however, are more highly hydrolyzed (28, 282), as would be expected. Jones and his coworkers (203, 204), in their accurate conductivity and transference studies upon lanthanum chloride solutions, found it unnecessary to correct for hydrolysis, and inasmuch as europic chloride and nitrate solutions exhibit the same conductances as the corresponding lanthanum salt solutions at the same concentrations (220), it would appear that appreciable hydrolysis is also ruled out for europium. As a matter of fact, the recorded conductivities for yttrium and rare earth salts are so nearly the same at equivalent concentrations that if hydrolysis is negligible in one instance it must be very nearly so in others.

Considerable emphasis has been placed upon the closeness of approach of experimental data to the Ostwald-Walden relation (28, 59, 60, 200, 242, 281)

$$\Lambda_{1024} - \Lambda_{32} = Cn_1n_2$$

where Λ_{1024} and Λ_{32} = equivalent conductances at dilutions of 1024 and 32 liters, respectively,

n_1 and n_2 = valences of cation and anion, respectively, and

C = a constant with a value very nearly 10.

Thus for salts of the type RX_3 , this conductivity difference should approach 30 (actually, it averages about 26), whereas for those of the type $R_2(SO_4)_3$ it should approximate 60 (actually, 52). Positive deviations indicate hydrolysis.

Summarized in table 7 are data for conductivity differences at 25°C. collected from a number of sources. It is apparent that the degree of hydrolysis is appreciable only in the case of scandium (28). Among the other materials, the differences for a particular type of compound are so nearly the same as to preclude any very definite conclusions as to relative extents of hydrolysis. Bodländer (28) calculated that scandium chloride at a dilution of 1024 liters was only 4.59 per cent hydrolyzed but gave no data for the other materials.

Although most authors are agreed that significant hydrolytic differences are not detectable by this means, Brauner and Švagr (59, 60) have demonstrated some regularity. Their measurements upon solutions of the normal sulfates indicated lower conductivities at 1024 liters for freshly prepared solutions than for solutions prepared by dilution over a period of 4 hr. These were ascribed to lessened hydrolysis in the freshly prepared solutions, and the differences between the two conductivities were given as a kind of measure of hydrolysis. These differences were given as: samarium, 1.80; lanthanum, 1.94; praseodymium, 3.35; gadolinium, 3.41; neodymium, 3.44; ytterbium, 4.45; yttrium, 4.89; cerium, 5.67. The high value for cerium was ascribed to partial oxidation of the cerous compound, and increases in hydrolysis were regarded as paralleling increases in these differences. The conductivity of scandium sulfate solution was found to change continually at higher dilutions because of hydrolysis, and the sulfates of terbium,

erbium, and ytterbium were stated to be highly hydrolyzed in extremely dilute solutions.

TABLE 7

Conductivity differences between dilutions of 1024 and 32 liters at 25°C.

ELEMENT	RCl_3	RBr_3	RI_3	$\text{R}(\text{NO}_3)_3$	$\text{R}(\text{C}_2\text{H}_3\text{O}_2)_3$	$\text{R}_2(\text{SO}_4)_3$	$\text{R}_2(\text{SO}_4)_3$ (fresh)
Sc.....	38.19 (28) 37.8 (282)					43.85 (28) 52.36 (60)	
Y.....	26.12 (28) 25.26 (200) 25 (248)	26.20 (200)	26.41 (200)			53.45 (28) 53.94 (59, 60)	49.05 (60)
La.....	26.84 (28) 25.7 (248) 25.8 (307) 25.8 (355)			28 (307)	42.3 (248)	51.87 (28) 53.65 (59, 60) 47 (307)	51.71 (60)
Ce....	26.81 (28)				41.8 (248)	51.95 (28) 55.66 (59, 60)	49.99 (60)
Pr.....	26.54 (28)					52.22 (28) 53.56 (59, 60)	50.21 (60)
Nd.....	26.06 (28)					53.87 (28) 53.35 (59, 60)	49.91 (60)
Sm.....	27.19 (200) 31.2 (355)	24.03 (200)	22.59 (200)			52.80 (28) 51.15 (59, 60)	49.35 (60)
Gd.....	28.19 (200)	23.53 (200)	21.18 (200)			50.85 (28) 51.54 (59, 60)	48.13 (60)
Tb.....						51.63 (59, 60)	
Er.....	25.73 (200)		20.72 (200)			54.25 (28) 54.73 (59, 60)	
Yb.....	26.37 (200) 33.0 (355)	24.73 (200)				55.34 (59, 60)	60.98 (60)

Brauner and Švagr (59, 60) also compared the conductivities of solutions containing equivalent quantities of the normal sulfates and sulfuric acid at dilutions of 32 liters with the conductivities of solutions of the sulfate and sulfuric

acid solutions alone. Expressing their results as per cent loss in conductivity of the mixed solution as compared with additive values for the components alone, they obtained: samarium, 6.7 per cent; terbium, 6.8 per cent; lanthanum, 7.8 per cent; cerium, 8.0 per cent; erbium, 8.0 per cent; neodymium, 9.7 per cent; gadolinium, 9.7 per cent; ytterbium, 9.9 per cent; praseodymium, 10.0 per cent; yttrium, 11.0 per cent. While they believed that a parallel should exist between increased conductivity loss and increased negativity of the metal, the many inconsistencies seemed to preclude such a conclusion. These inconsistencies were ascribed to difficulties in obtaining constant conductivity values for the sulfuric acid solutions (60).

TABLE 8
Hydrogen-ion concentration data for aqueous salt solutions

CATION	CHLORIDES— <i>N</i> /10 (28)			NI- TRATES <i>N</i> /100 (314) pH	CHLORIDES (214) pH		MISCELLANEOUS		
	[H ⁺] × 10 ⁶	pH	Hydroly- sis		<i>N</i> /10	<i>N</i> /100	pH	Remarks	Refer- ence
	<i>gram-moles per liter</i>		<i>per cent</i>						
Sc ⁺⁺⁺	896	3.05	0.896				4.93	<i>M</i> /100 C ₂ H ₃ O ₂ ⁻	(388)
Y ⁺⁺⁺	10.43	4.98	0.0104		1.0	1.3			
La ⁺⁺⁺	3.26	5.49	0.00326	6.605	3.7	5.0	6.2 6.2	Concentrated Cl ⁻ <i>M</i> /10 Cl ⁻	(204) (222)
Ce ⁺⁺⁺	5.28	5.28	0.00528	5.682	1.8	2.5			
Ce ⁺⁺⁺⁺				2.65					
Pr ⁺⁺⁺	4.27	5.37	0.00427	4.238	3.1	4.5			
Nd ⁺⁺⁺	4.94	5.31	0.00494	4.526	2.5	3.2			
Sm ⁺⁺⁺	7.62	5.12	0.00762	"	1.4	2.1			
Gd ⁺⁺⁺	6.20	5.21	0.00620						
Dy ⁺⁺⁺	12.2	4.91	0.0122		5.8	6.8			
Ho ⁺⁺⁺						1.0			
Er ⁺⁺⁺	15.4	4.81	0.0154		1.1	1.7			
Th ⁺⁺⁺⁺				2.435					

(2) Hydrogen-ion concentrations in aqueous salt solutions

Inasmuch as the hydrolysis of metal salts in aqueous solution alters the hydrogen(hydronium)-ion concentration to values different from that existent in pure water, measurements of hydrogen-ion concentration represent quantitative approaches to hydrolysis. Only a very few measurements of this type have been reported for scandium, yttrium, and the rare earth elements.

Bodländer (28) determined hydrogen-ion concentrations in *N*/10 and *N*/32 solutions of the trichlorides of scandium, yttrium, lanthanum, cerium, praseodymium, neodymium, samarium, gadolinium, dysprosium, erbium, and neoytterbium at 25°C. by means of a hydrogen electrode. The data obtained for *N*/10 solutions are listed in table 8, together with the percentages of hydrolysis

given in the original dissertation and pH values calculated from the listed hydrogen-ion concentrations. While the percentages of hydrolysis are small for all materials except scandium and not subject to much variation, the corresponding pH values decrease in the series

La, Pr, Nd, Ce, Gd, Sm, Y, Dy, Er, Sc

and this may be taken as the order of decreasing basicities (28). In $N/32$ solution the order is

La, Pr, Gd, Ce, Nd, Sm, Y, Er, Dy

Although Bodländer believed the differences among the various materials to be within experimental error (28), both she and Meyer (283), in reporting her results, were of the opinion that the data indicated basicity decreases in the series lanthanum to europium and gadolinium to thulium, with increases between europium and gadolinium and following thulium. This is in conformity with the observations of Brauner (55) and Brauner and Švagr (60).

Neish and Burns (314) employed a hydrogen electrode to measure the hydrogen-ion concentrations in several $N/100$ solutions at 25°C . Their data are also given in table 8 and are in good agreement with those of Bodländer. The order of increasing hydrolysis and decreasing basicity from these data is lanthanum, cerium(III), neodymium, praseodymium, cerium(IV), thorium. Neish and Burns believed lanthanum hydroxide to be a stronger base than ammonium hydroxide and indicated the basicities of praseodymium and neodymium to be so nearly the same as to preclude use of basicity methods for their separation.

The pH values of solutions prepared by dissolving a number of anhydrous chlorides in water were determined at 25°C . by Kleinheksel with Kremers (214) by use of a hydrogen electrode. Values interpolated from their data to $N/10$ and $N/100$ solutions are listed in table 8. Except for dysprosium, these values are considerably lower than those reported by other workers and would thus indicate rather extensive hydrolysis. The use of anhydrous chlorides may have contributed to these low values. The order of decreasing basicity was given by these authors as dysprosium, lanthanum, praseodymium, neodymium, cerium(III), samarium, thulium, yttrium, holmium. The anomalously high basicity of dysprosium was unexplained, although the material was stated to be pure.

In some early measurements, Denham (115) found the hydrogen-ion concentration in cerous chloride solutions to vary from day to day, the hydrolysis of the material at a dilution of 32 liters amounting to 0.14 per cent.

A few additional values for hydrogen-ion concentration obtained from various sources are also included in table 8. It is apparent that the available data are very fragmentary and perhaps of doubtful validity. More comprehensive investigations are indicated. General decreases in basicity beyond lanthanum are indicated, with yttrium occupying some intermediate place in the rare earth series and scandium being the least basic of all.

(3) Viscosities of aqueous salt solutions

Use of measurements of viscosity as a means of determining extent of hydrolysis has been reported by Tollert (396). On the basis of measurements of specific viscosities of solutions of trivalent cerium earth metal nitrates and ceric sulfate alone and in the presence of added nitric and sulfuric acids, respectively, Tollert indicated that if the concentrations corresponding to equal viscosities for rare earth salt solution and rare earth salt solution plus added acid be plotted against atomic numbers, a regular decrease from lanthanum to praseodymium occurs, paralleling a decrease in basicity. With neodymium and samarium, slight increases occurred.

A further measure of hydrolysis effects was obtained through evaluation of the percentage deviation of the measured specific viscosity for the rare earth ion from the calculated value, which percentage in turn gave the percentage deviation in hydrolysis in the absence of acid from that in the presence of acid at a particular salt to acid ratio (1:1). These deviations decreased from lanthanum to praseodymium and then increased slightly to samarium, the value reported for neodymium being somewhat high because of impurities (396). Parallels between these deviations and reduction potentials (325), hydrolysis as determined by other methods, and basicity were noted.

4. Standard electrode potentials

While it is generally true that elements with strongly basic properties have high negative (convention employed by Kolthoff, etc.) standard electrode potentials, the variety of factors summed up in the electrode potential makes the magnitude of this quantity alone of doubtful utility in expressing basicity. Thus, Latimer (239) indicates that no direct relation exists between the oxidation potential of a metal and the basic or acidic properties of its oxide.

Reliable data for the standard electrode potentials (E^0) for scandium, yttrium, and the rare earth metals are very limited. Kapustinskii (208) has determined the standard potentials at 25°C. from thermal data, and Latimer (240) has listed approximate values at 25°C. for scandium, yttrium, and lanthanum. These data are summarized in table 9, the sign convention used by Kolthoff and Lingane (224) being employed.

On the basis of polarographic measurements at 20°C., Noddack and Brukl (325) arrived at two series of potential values referred to the normal calomel electrode. These series were attributed respectively to reduction of the trivalent ions to the divalent (E_1) and reduction of the divalent ions to the free metals as amalgams (E_2). These values are listed in table 9. The stepwise reduction of the trivalent elements as proposed has been objected to by both Heyrovsky (166) and Kolthoff and Lingane (224), who contend that the first of these potentials doubtless refers to the reduction of the hydrogen ion in all cases except with europium.

Noddack and Brukl (325) pointed out that since their data represented measures of the work necessary to form amalgams from the ions, the potentials should measure basicities, corrections for the energies of amalgamation being only very

small and exactly similar in all cases. The proposed basicity series was then obtained from comparisons of the values of $E_1 + 2E_2$, as listed in table 9. Basicities thus decreased in order from lanthanum through lutecium, with yttrium falling between holmium and erbium and scandium following lutecium. On the basis of the relative basicities so indicated, Noddack and Brukl classified the rare earth elements into three groups: lanthanum through neodymium, samarium through gadolinium, and terbium through lutecium. Klemm (216, 217) has attacked this arrangement on the ground that it is not in agreement with arrangements based upon other data.

While polarographically determined reduction potentials are not identical with electrode potentials, their values are roughly of the same order of magnitude (223). Using this as a basis, and accepting the assumption of Noddack and Brukl that their data do represent two reduction steps, one can calculate potentials for the direct reduction of the trivalent ions to the free metals (as amalgams) by means of the relation

$$E = (E_1 + 2E_2)/3$$

Values so obtained, as converted to the basis of the normal hydrogen electrode, are listed in table 9. These values are of the same order of magnitude as the standard potentials also listed and indicate increases from scandium through yttrium to lanthanum, followed by decreases.

An approach to a quantitative relationship between electrode potential and basicity has been made by Heyrovsky (165). Basing his considerations upon a thermodynamic treatment of the various factors contributing to the electrode potential and upon the chemical affinity of a metal as related to its mass, Heyrovsky derived the expression

$$-B = E - kT \log m + k'T - K$$

where

B = basicity of the hydroxide of the metal,

E = electrode potential,

T = temperature in degrees Kelvin,

m = mass of the cation, and

k, k', K = constants.

Since at room temperature the value of kT is nearly 1.7, substitution, combination of the constants, and transposition lead to

$$B + K' = 1.7 \log m - E$$

and basicity can be calculated from cation mass and electrode potential. The utility of this expression in correctly evaluating the basicities of a number of elements has been shown (165).

Values calculated through application of this expression to electrode-potential data are listed in table 9. The expected increase from scandium through yttrium to lanthanum is apparent. Beyond lanthanum, the differences in potentials are

insufficient to overcome the mass differences, and the calculated basicities are meaningless. They do serve to indicate the great similarity in basic properties among the rare earth elements.

TABLE 9
Electrode potentials and basicities

ELEMENT	KAPUSTINSKII (208)		LATIMER (240)		NODDACK AND BRUEL (325)				
	E^0	$1.7 \log m - E^0$	E^0	$1.7 \log m - E^0$	E_1	E_2	$E_1 + 2E_2$	E	$1.7 \log m - E$
	volts		volts		volts	volts	volts	volts	
Sc.....			-2.0	4.81	-1.630	-1.790	-5.210	-2.019	4.83
Y.....	-1.88	5.19	-2.1	5.41	-1.795	-1.880	-5.555	-2.134	5.45
La.....	-2.42	6.06	-2.37	6.01	-1.935	-2.040	-6.015	-2.287	5.93
Ce.....	-2.40	6.05			-1.905	-2.010	-5.925	-2.257	5.91
Pr.....	-2.47	6.13			-1.875	-1.990	-5.855	-2.234	5.89
Nd.....	-2.38	6.05			-1.870	-1.960	-5.790	-2.212	5.88
Sm.....	-2.32	6.02			-1.720	-2.010	-5.740	-2.195	5.90
Eu.....					-0.710	-2.510	-5.730	-2.192	5.90
Gd.....					-1.810	-1.955	-5.720	-2.189	5.92
Tb.....					-1.830	-1.925	-5.680	-2.175	5.92
Dy.....					-1.800	-1.905	-5.610	-2.152	5.91
Ho.....					-1.790	-1.885	-5.560	-2.135	5.90
Er.....					-1.770	-1.875	-5.520	-2.122	5.90
Tm.....					-1.770	-1.850	-5.470	-2.105	5.89
Yb.....					-1.430	-2.005	-5.440	-2.095	5.89
Lu.....					-1.755	-1.820	-5.395	-2.080	5.89

TABLE 10
Heats of solution of oxides in acids

OXIDE	HEAT OF SOLUTION	
	H ₂ SO ₄	HCl
	Calories	Calories
La ₂ O ₃	117.6	114.6
Pr ₂ O ₃	106.5	106.2
Nd ₂ O ₃	106.4	105.5
Sm ₂ O ₃	97.4	94.6

5. Thermochemical investigations

The heats of formation of compounds do not in general give exact measures of the basicities of the elements involved (260). Using data on the heats of combustion of some of the cerium group metals obtained by Muthmann and Weiss (311), Meyer and Hauser (285) have calculated the following heats of formation (expressed as calories per gram-equivalent of oxide): La₂O₃, 74.1; CeO₂, 56.1; Pr₂O₃, 68.7; and Nd₂O₃, 72.5. No general conclusions can be drawn from these values.

More direct thermochemical evidence of basicity variations can be obtained from the data of Matignon (268-273) on the heats of solution of some of the oxides in acids, as given in table 10. Decreasing values indicate a decrease in basicity in the series lanthanum, praseodymium, neodymium, samarium. The value for neodymium oxide is close to that for magnesium oxide (273).

6. Thermal decompositions of oxygen-containing salts

The ease with which an oxygen-containing salt can be converted into a basic salt or metal oxide by heating can be used to measure the basicity of the metal in question. The lower the temperature necessary for this conversion, the more strongly the electrons upon the acid group were attracted by the cation and the more readily that group was decomposed. Decreases in basicity should thus parallel decreases in decomposition temperature.

The relation between the temperatures at which the nitrates of these elements are decomposed and basicities has been recognized ever since the introduction of nitrate fusion as a method of separation (18). Although most of the experimental work on nitrate decompositions has been concerned with the separation of mixtures, it has been pointed out (32, 278, 379) that nitrates decompose in the order

Th, Ce(IV), Sc, Yb, Tm, Er, Ho, Tb, Y, Sm, Gd, Nd, Pr, La

Urbain (399) indicated separation by nitrate fusion to take place in the order

Sc, Yb, Er, Ho, Tm, Tb, Gd, Y

With a few inconsistencies, these orders agree with other arrangements of increasing basicities.

The thermal dissociation of the sulfates of a number of the trivalent elements has been investigated by Wöhler and Grünzweig (439) through measurement of sulfur trioxide pressures in the temperature range 800-1020°C. Basic sulfates were usually formed, although cerous sulfate gave ceric oxide and scandium sulfate decomposed completely to the oxide even at 780°C. In the order of decreasing pressures of sulfur trioxide at any particular temperature, the elements were arranged as follows:

Sc, Sm, Gd, Nd, Pr, Er, Yb, Lu, Y, La

At 900°C., the dissociation energies (in Calories) were as follows: cerium, 52.4; scandium, 54.5; samarium, 56.6; gadolinium, 56.9; neodymium, 57.2; praseodymium, 57.4; erbium, 57.6; ytterbium, 58.2; lutecium, 58.5; yttrium, 58.9; lanthanum, 59.8. The indicated order of basicity differs from that obtained by any other method. The authors pointed out that although the rare earths are comparable in basic strength to the alkaline earths, the cerium earths are less basic and the yttrium earths more basic than usually supposed. Such conclusions are questionable.

In a subsequent paper, Wöhler and Flick (438) reported the following dissociation temperatures for the anhydrous sulfates: lanthanum, 1050°C.; neo-

dymium, 1021°C.; praseodymium, 1011°C.; cerium(III), 950°C.; cerium(IV), 490°C. Basicities may be regarded as decreasing in this order, although most of the differences are not particularly significant.

Willard and Fowler (433) heated sulfate mixtures to carefully controlled temperatures and found cerous sulfate to be decomposed more readily than the lanthanum, praseodymium, and neodymium compounds. No attempt was made to establish relative basicities, and the lowered decomposition temperature of the cerous material was doubtless influenced by oxidation to the ceric.

In investigating the thermal decompositions of the normal carbonates of the cerium group elements, Preiss and Rainer (348) found cerous carbonate to yield basic ceric carbonates and finally ceric oxide, whereas the others gave basic carbonates of the types $R_2O_3 \cdot 2CO_2$ and $R_2O_3 \cdot CO_2$. Dissociations yielding the latter type were regarded as true measures of basicities and placed these elements with calcium in basicity. Decomposition of these basic carbonates to the sesquioxides occurred at the following temperatures: lanthanum, 900°C.; praseodymium, 815°C.; neodymium, 800°C. This order of stability measures relative basicities and is the same as that obtained for the hydrolysis of the carbonates (347).

According to Somiya and Hirano (377), the temperatures at which the basic carbonates of the rare earth elements decomposed to oxides increased with increasing basicity of the metal present. The temperatures at which basic carbonates obtained from the oxalates decomposed increased in the order samarium, neodymium, praseodymium, lanthanum. Experiments involving the decomposition of nitrates in atmospheres of carbon dioxide indicated that temperatures corresponding to the conversions $R(NO_3)_3$ to $RONO_3$ and $R_2O_3 \cdot CO_2$ increased in the same order. Cerous oxalate and nitrate decomposed at lower temperatures because of oxidation.

Kato (210) found the limiting temperatures necessary for the complete conversions of acetates to oxides to be as follows: cerium(IV), 600°C.; lanthanum, 760°C.; didymium, 800°C. The corresponding values for nitrates were: ytterbium, 590°C.; erbium, 680°C.; praseodymium, 690°C.; neodymium, 720°C.; lanthanum, 745°C.; and cerium(IV), 830°C. With the exception of the excessively high value for cerium, the results can be regarded as indicative of a basicity increase in this order.

C. SUMMARY

A summary of existing information pertaining to relative basicities of the trivalent materials was published in 1933 by Sherwood with Hopkins (374). Results based upon a number of experimental approaches indicated the materials to be grouped into three zones. Zone 1, containing the cerium earths and possibly yttrium, was regarded as definitely established, with basicities decreasing with increasing atomic weight or number. Zone 2, containing samarium, europium, and gadolinium, was regarded as unsettled because of a variety of reports on the relative basicities of these elements. Zone 3, containing the remainder of the elements, was considered as settled, with basicities decreasing

regularly from terbium through lutecium, and with scandium and cerium(IV) following lutecium.

As has already been pointed out, uncertainties in the relative basicities of samarium, europium, and gadolinium have been eliminated by more precise experimental measurements (155, 374, 297), and the order in Zone 2 can now be regarded as definite as that in either of the other two zones. In table 11, more

TABLE 11
Orders of decreasing basicities

A	B	C	D	E	F	G	H	I	J	K	L	M	N	O	P
La	La	La	La	La	La		La	La	La	La	La	Dy	La	La	La
Ce	Ce		Ce	Pr	Ce		Ce		Pr		Ce	La	Ce		
Pr	Pr	Pr	Pr	Ce		Pr	Gd	Pr	Nd	Pr	Pr	Nd	Pr	Pr	Pr
Nd	Nd	Nd	Nd	Nd	Nd	Nd	Pr	Nd	Ce	Nd	Nd	Ce	Nd	Nd	Nd
Sm	Y		Sm	Sm	Sm	Sm	Nd	Y	Y	Ce		Sm	Sm	Sm	Gd
Eu	Sm		Eu	Eu	Eu	Eu	Y	Y	Sm	Gd		Tm	Eu		Sm
Gd	Eu	Gd	Gd	Gd	Gd	Gd	Sm	Sm	Eu	Sm			Gd		Y
Tb	Gd					Tb	Tb	Eu	Tb	Y			Tb		Tb
Dy	Tb	Y				Dy		Gd	Gd	Dy			Dy		
Y	Dy	Dy	Y	Y		Y		Tb	Dy			Y	Ho		
Ho	Ho						Er	Dy	Ho			Ho	Y		Ho
Er	Er		Er	Er	Er			Ho	Er	Er			Er		Er
Tm	Tm		Tm	Tm		Tm		Er	Tm				Tm		Tm
Yb	Yb		Yb	Yb	Yb	Yb		Tm	Yb				Yb		Yb
Lu	Lu		Lu	Lu				Yb					Lu		Sc
Sc	Sc		Sc	Sc				Lu	Sc	Sc			Sc		
Th			Th												
Ce*	Ce*		Ce*						Ce*		Ce*	Th			Ce*

* Tetravalent cerium.

A = theoretical (392, 393, 411-414).

B = ammonia precipitation (33, 155, 241, 278, 379, 401).

C = solubility in buffers (133).

D = precipitation pH (44, 64, 65, 297, 327, 361, 389).

E = solubility product and solubility (240, 297, 327, 361).

F = sulfate hydrolysis (211).

G = carbonate hydrolysis (63).

H = sulfate hydrolysis (59, 60).

I = nitrite hydrolysis (185, 373, 374).

J = azide hydrolysis (2).

K = pH of chloride solutions (28).

L = pH of nitrate solutions (314).

M = pH of chloride solutions (214).

N = electroreduction (325).

O = heat of solution of oxides (268-273).

P = nitrate decomposition (32, 278, 379).

complete summaries of the orders of decreasing basicities based upon a variety of approaches are listed. While a number of inconsistencies appear in these arrangements, the bulk of the experimental evidence supports the order deduced theoretically. The anomalously high basicity of yttrium as obtained by measurements upon natural mixtures is undoubtedly a concentration effect ascribable to the relatively great abundance of that element.

III. SEPARATIONS BASED UPON DIFFERENCES IN BASICITY

Although the basicities of most of these elements in the positive three state of oxidation differ but little, significant differences existent between the two ends of the series permit at least preliminary separations in this fashion. The most basic member, lanthanum, and the least basic, scandium, can often be obtained in relatively pure condition by methods based upon differences in basicity. In addition, basicity methods are often employed to effect preliminary separations among the members of the yttrium subgroup after this subgroup has been separated from the cerium subgroup by some other procedure. When used for separations in the yttrium subgroup, basicity methods are most effective for the partial removal of yttrium (247, 404) but are not effective in rapidly separating other elements, particularly those in the region of terbium (245). The apparently high basicity of yttrium in natural mixtures is used to advantage in such procedures.

The reduced basicity of the positive four oxidation state can be employed for the separation of cerium from the trivalent elements by basicity procedures. The low basicity of tetravalent thorium is also used to advantage in the removal of this element from rare earth ores or mixtures.

Inasmuch as most basicity procedures involve fractional precipitation or dissolution, they are apt to be experimentally tedious and may be wasteful of material (190, 338). For these reasons, they have not been as extensively employed as many of the crystallization procedures (343). However, for certain specific separations, they can be relatively efficient and can thus be employed to advantage in conjunction with the slower crystallization methods in general separations (243), especially if the latter methods are no longer capable of producing results (343).

It must be pointed out, however, that any attempt to separate by a basicity method two elements which differ only slightly from each other in basicity is highly impractical and may take an almost infinite length of time. If two materials of nearly the same basicity are to be separated by fractional precipitation, the smaller the quantity of the precipitate in each step, the greater the ratio of the amount of the less basic component to the more basic one therein (177). Reduced to practical terms, this means that because only small amounts can be precipitated each time, tremendously large amounts of material would be necessary to give any sort of efficiency to the process.

A quantitative treatment of the course of such a separation in terms of the relative basicities of the elements concerned was worked out by Crookes (95). This has been summarized by Mellor (275), and amounts merely to pointing out that precipitation of the materials by some basic substance such as ammonia is directly dependent upon the excess of basicity of the precipitant over that of a material being precipitated. Thus, if the two materials of relative basicities 100 and 101 were treated with ammonia of relative basicity 150, the course of precipitation would be represented by $150 - 100 = 50$ and $150 - 101 = 49$, with a separational difference of one part in fifty, or 2 per cent.

A number of excellent summaries of separational procedures based upon differences in basicity have appeared (32, 180, 193, 252, 253, 254, 255, 256, 274, 279, 280, 371, 379, 380, 381, 399, 400, 417). The comparative efficiencies of a number of such procedures have been discussed qualitatively (371), but the only quantitative study of this type appears to be one on the relative efficiencies of several methods for the separation of neodymium from lanthanum (370). In the treatment which follows, general information relative to a number of basicity procedures is summarized.

A. PRECIPITATION BY ALKALINE MATERIALS

In a sense, any method of fractional precipitation can be regarded as depending upon differences in basicity. However, since there exists no simple relationship between the relative attractions for electrons and solubilities (155), there appears to be no direct connection between the orders in which many compounds of these elements precipitate and their basicities. Consequently, only those precipitation procedures which are directly dependent upon hydroxyl or hydrogen ions are included in this discussion.

1. *Caustic alkalis*

Although sodium and potassium hydroxides appear as logical precipitants, the high concentrations of hydroxyl ion existent even in dilute solutions of these materials make it difficult to avoid the undesirable effects of localized excesses when these reagents are added to mixed salt solutions. As a consequence, few workers have employed them, although Drossbach (129, 130) believed sodium hydroxide to be superior to ammonia, since the precipitated hydrous oxides or hydroxides dissolve to some extent in the accumulated ammonium ion. The early literature has been completely reviewed by Böhm (32).

Caustic alkalis appear to be most useful for removing traces of less basic materials from larger amounts of more basic ones. Thus, Drossbach (129) readily removed didymium from lanthanum by precipitation of the former with sodium hydroxide. Similarly, Brauner and Pavliček (57) used potassium hydroxide to prepare lanthanum material of atomic weight purity. Holden and James (172) boiled yttrium-erbium mixtures with sodium hydroxide, precipitating one-fifth of the total in each fraction, and removed erbium in the less basic fractions. Willand and James (432) achieved some success in the same separation by the use of sodium hydroxide in the presence of tartaric acid. Bowles and Partridge (44) separated ceric material from lanthanum by adding sodium hydroxide to a mixed sulfate solution in sulfuric acid to a pH of 5.78 and filtering off the precipitated ceric compound. On the other hand, Baskerville and Turrentine (11) were unable to separate neodymium from praseodymium with caustic alkali, the basicities of these two materials being too nearly the same.

2. *Metal oxides and carbonates*

The highly basic magnesium oxide has long been employed in fractional separations, particularly for the removal of less basic earths from lanthanum. Thus,

Bunsen (73) removed most of the didymium from lanthanum by precipitating the former with magnesia, although Muthmann and Rölig (310) are generally given credit for developing the process (279, 379). These authors prepared relatively pure lanthanum oxide, although some lanthanum precipitated with the didymium. The use of magnesium oxide for this separation has been recommended by a number of workers (131, 194, 290, 314, 370, 430), and Drossbach (129, 130) and James (192) have applied it to the separation of the less basic members of the yttrium subgroup. The method is always fractional in character but is convenient (430) and relatively efficient (370, 430). Experimentally, it amounts merely to the addition of either magnesium oxide paste or powder to a boiling neutral salt solution followed by filtration to remove the first-precipitated, least basic members.

Various other metal oxides have been employed. Thus, de Boisbaudran (103) found that cuprous oxide would precipitate thorium from rare earth salt solutions, the simultaneous precipitation of ceric material being prevented by prior reduction to the more basic cerous condition. Other investigators have employed cuprous oxide (368, 399), but its use has not become general. Smith and Heyl (375) reported partial precipitation of cerous and lanthanum hydroxides by mercuric oxide from cold solution and complete precipitation from hot solution but attempted no separations. Zinc oxide was investigated by Witt and Theel (437), but it appears to be most useful in the precipitation of ceric material after oxidation of the cerous (191, 209, 292, 310, 356, 357, 391, 435). For this purpose, zinc oxide effectively removes ceric ion from the more basic trivalent ions and is preferred to magnesium oxide (292) and mercuric oxide (435).

In an attempt to find specific metal oxides or carbonates for the precipitation of certain of the elements, Neish and Burns (314) measured the hydrogen-ion concentrations in 0.01 *N* nitrate solutions of lanthanum, trivalent and tetravalent cerium, praseodymium, neodymium, and thorium at 25°C. and compared the results with the hydroxyl-ion concentrations produced by creams of various insoluble metal oxides and carbonates. On the basis of the quantity of hydrogen ion in the salt solution and the quantity of hydroxyl ion in contact with the oxide or carbonate, they predicted that:

1. Certain oxides, such as FeO and CoO, furnishing only a small amount of hydroxyl ion, would not be expected to precipitate thorium completely.
2. Other oxides, such as CdO and HgO, furnishing more hydroxyl ions, would precipitate thorium completely.
3. Other oxides and carbonates, such as ZnO, CuO, PbO, Pb₂O₄, and PbCO₃, furnishing still more hydroxyl ions, would precipitate thorium and ceric cerium completely but would not precipitate the trivalent materials.
4. Such materials as Ag₂O, MgO, and MgCO₃, which furnish high concentrations of hydroxyl ion, would be needed to separate praseodymium and neodymium from lanthanum.
5. Since lanthanum hydroxide is a stronger base than ammonium hydroxide, such a material as NaOH would be required to precipitate it completely.

On the basis of these predictions and a number of experimental separations,

the following scheme of analysis was proposed (314): After reduction of cerium, thorium is precipitated completely with lead carbonate, zinc carbonate, calcium carbonate, lead oxide (Pb_2O_4), or zinc oxide. Cerium is then oxidized in the filtrate and precipitated by any of these same carbonates or oxides. The resulting cerium-free filtrate is then treated with argentous oxide, magnesium oxide, or magnesium carbonate at 60°C . to precipitate praseodymium and neodymium, and lanthanum is recovered from the final filtrate by precipitation with sodium hydroxide. A futile attempt was made to separate neodymium from praseodymium by ammonia precipitation, but the authors stated that such a separation could be made in time. Comparable systematic studies upon the remaining elements should go far toward systematizing many highly empirical precipitation procedures.

3. Rare earth oxides or hydroxides: the "oxide processes"

Inasmuch as the various rare earth elements and yttrium differ from one another in basicity, the use of oxides or hydroxides of the more basic elements to precipitate the less basic ones suggests itself. A number of so-called "oxide processes" based upon this principle have been employed. Often they are all referred to in general as Welsbach's oxide process. Details of these procedures have been summarized in several discussions (254, 256, 279, 280, 379, 425).

The general method was first proposed by Hermann (163), who precipitated mixed lanthanum and didymium oxides and added them to a new mixed nitrate solution, the lanthanum oxide dissolving and precipitating out only didymium. Later Auer von Welsbach (423) proposed the procedure as a general one for the precipitation of less basic materials by more basic ones. The method has been used with considerable success in separating didymium from lanthanum (29, 58, 129, 131, 310, 311, 364, 425) and in separating the yttrium earths and yttrium from the yttrium earths (25, 132, 424, 425, 429). Drossbach (132) reported an excellent separation of erbium from yttrium by digesting a chloride solution with oxide at elevated temperatures. Wichers, Hopkins, and Balke (429), however, reported this separation to be much less rapid than Drossbach had indicated. This method can be used to effect complete removal of cerium (425).

For the separation of lanthanum, a portion of the strongly ignited oxides is stirred to a paste with water and then added to a neutral nitrate solution prepared from an equal weight of the original oxide and free from ammonium salts. After standing, the suspension is heated on the water bath for some hours, allowed to settle, and filtered. Both precipitate and mother liquor are then reworked in the same fashion (254, 279, 379).

For the yttrium earths, the oxide mixture is ground to a paste with water and about one-half is dissolved in nitric acid. Small amounts of the paste and nitric acid are then added successively to the nitrate solution, care being taken that at no time does all of the oxide dissolve. After all the paste has been added, the mixture is allowed to cool, whereupon the less basic elements precipitate as basic nitrates. Sufficient nitric acid is then added to dissolve the remaining oxide, and the mass is allowed to stand. The less basic materials are found as in-

soluble basic nitrates, while the more basic ones, especially yttrium, remain as soluble normal nitrates which can be removed with water or alcohol (280, 379). In this form, the oxide process involves not only basic precipitation but also hydrolysis.

4. Ammonia

Fractional precipitation with ammonia has long been a favorite procedure (32). Thus, it has been employed for the purification of lanthanum (46, 52, 82, 83, 84, 88, 135, 143, 370); for the separation of the cerium earths in general (89, 105, 106, 109, 339, 340, 342, 397, 437); for the separation of the yttrium earths in general (19, 95, 96, 104, 107, 118, 170, 261, 266, 308, 309, 332, 335, 336, 337, 397, 405); and for such specific separations as gadolinium from samarium (16, 24, 109, 155, 261), holmium from erbium (25, 174, 183), didymium from cerium (49), didymium from lanthanum (83, 86, 129, 143, 176), samarium from didymium (90, 102), yttrium earths from yttrium (107, 108, 118, 182, 397), other earths from erbium (168), dysprosium from terbium (193), terbium from gadolinium (291, 402), and praseodymium from lanthanum (430, 431). Reports that it is unsatisfactory for the purification of samarium (110, 111) and for the separation of neodymium from praseodymium (263, 343) have also appeared, although Cleve (86) felt that his results with the method indicated some complexity of didymium. As a variation, the use of anhydrous ammonia in acetone has been suggested (8).

Almost all investigators have been favorably impressed with the general aspects of the method, although its limitations must be recognized. The purification of lanthanum and of yttrium represents its major use, and in no case can it be used to advantage unless preceded by other processes which effect preliminary separations.

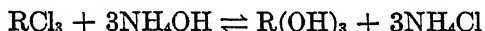
In general, ammonia fractionation consists of adding a dilute solution of aqueous ammonia to the mixed salt solution, either hot or cold, followed by filtration after an appropriate interval of digestion and repetition of the process (379). This simple procedure may lead to one or the other of the following difficulties: gelatinous, hard to filter, and strongly adsorbing precipitates, or localized excesses of reagent which lead to precipitation in an order different from that of increasing basicities. Avoidance of the first difficulty is commonly effected by digestion at elevated temperatures (280). Avoidance of the second may be effected in a variety of fashions.

Thus, excessive dilution of either reactant may result in sufficiently slow precipitation of the hydrous material to avoid local excesses. Hopkins and Balke (182) found that excessively dilute ammonia could be added in its entirety to yttrium earth chloride solutions before precipitation occurred. Upon standing, such solutions slowly deposited precipitates. Hopkins and Kremers (183) added 0.01 *N* ammonium hydroxide to solutions containing yttrium, holmium, and erbium chlorides, and found that precipitates were formed several hours after mixing. Such a method is slow because the quantity of precipitate is always small (343), but the ultimate results are excellent.

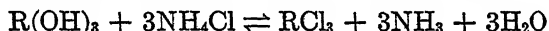
A recent innovation by Trombe (397) involves bubbling air through dilute aqueous ammonia and then through the rare earth salt solution. In this fashion accurate pH control is maintained without excessive dilution or the introduction of foreign materials. Excellent results were obtained, especially in the separation of the cerium and yttrium subgroups and in the elimination of yttrium.

An interesting series of investigations involving control of the hydroxyl-ion concentration in ammoniacal solutions through the use of ammonium salts and salts giving cations which form complex amines has been carried out by Prandtl and his coworkers (335, 336, 337, 339, 340, 342, 343, 344, 345, 346). These investigations were based upon the thesis that if the solubility of a hydrous hydroxide in the mother liquor were increased, the absolute solubility differences between neighboring materials would be magnified, and the hydroxyl-ion concentration could be so controlled that even in concentrated solutions only the least soluble material would separate (343).

Prandtl and Rauchenberger (343) pointed out that in precipitations with ammonia equilibria of the type



are set up and that in the presence of sufficient added ammonium salt precipitation with ammonia can be largely or even completely inhibited. In order then to determine experimentally the differences existent among the various rare earth elements, these authors studied equilibria of the type



by determining the quantities of rare earth material and ammonia present at equilibrium in ammonium chloride solutions of varying concentrations (1-5 *N*) and at temperatures of 15°, 30°, 50°, and 100°C. Such equilibria were approached from both directions, and the results were expressed graphically as plots of rare earth and ammonia concentrations against temperature for lanthanum, praseodymium, and neodymium.

In all cases, maximum solubilities were reached in 3 *N* ammonium chloride, with solubilities under a given set of conditions decreasing in the order lanthanum, praseodymium, neodymium. At 50°C. in 2-3 *N* ammonium chloride, lanthanum hydroxide was much more soluble than the other two hydroxides, while at more elevated temperatures the solubilities of all three approached one another as basic salt formation became more pronounced. At 50°C. and in 2-3 *N* ammonium chloride, lanthanum was readily and completely separated from relatively large amounts of praseodymium and neodymium. However, only small changes in composition were effected in praseodymium-neodymium mixtures. Precipitation of only about 5 per cent of the oxides in any one operation was recommended, because of strong adsorption of dissolved materials by the hydrous precipitates.

In a second paper (344), these observations were extended to samarium, the hydroxide of which was found to be less soluble in ammonium chloride solutions than that of neodymium, although not enough to permit the interposing of ele-

ment 61. Similar measurements were made for these same elements in ammonium nitrate solutions (1-5 *N*) at the same temperatures. Although the order of solubilities remained the same (decrease from lanthanum through samarium), solubilities increased with both increase in temperature and increase in ammonium nitrate concentration, the difference between lanthanum and praseodymium becoming so large that at 100°C. and in 4-5 *N* ammonium nitrate lanthanum could be readily separated from the other cerium earths by ammonia precipitation. Differences due apparently to alteration in the nature of the anion were ascribed to differences in the precipitation of basic salts.

Prandtl and Rauchenberger (344) proposed further that solubilities might be further influenced by the presence of cations which would bind a portion of the ammonia as ammine complex ions. Lanthanum, praseodymium, neodymium, and samarium nitrate solutions were treated with equivalent amounts of magnesium and zinc nitrates and ammonium nitrate (1-5 *N*) and precipitated with ammonia at 15°, 30°, 50°, and 100°C., solubilities being determined as before. Magnesium ion was without appreciable effect because of its inability to tie up free ammonia. In solutions containing zinc ion, however, while solubilities again decreased in the same order as before, the solubility of the lanthanum material was increased to about four times those of the others at 100°C. Separation of lanthanum in the presence of zinc and ammonium nitrates was efficient, but the solubilities of the other materials were so nearly the same as to prevent separation by ammonia precipitation.

Similar measurements upon lanthanum, praseodymium, neodymium, and samarium nitrate solutions containing ammonium and cadmium nitrates (345) indicated an even greater effect than with zinc and a feasible separation of lanthanum from the other cerium earths in boiling 2-3 *N* ammonium nitrate solution containing cadmium nitrate.

These observations were then extended to separations of the cerium-free materials of the cerium group (342). A nitrate solution containing the equivalent of 570 g. of mixed oxides was treated with ammonium and cadmium (3Cd^{++} to 2R^{+++}) nitrate solutions and precipitated with ammonia while being boiled and stirred. Use of 1 per cent ammonia in the initial precipitations yielded dense, easily filterable precipitates. Each precipitate was redissolved and further fractionally precipitated, the course of the fractionation being followed by absorption-spectra measurements. Samarium, neodymium, and praseodymium were readily removed, and relatively pure lanthanum was obtained. A somewhat greater separation of neodymium from praseodymium than previously reported by basicity methods was effected.

The preparation of pure praseodymium material from lanthanum-praseodymium concentrates was readily carried out by systematic ammonia precipitation in the presence of ammonium and cadmium nitrates (340), and an extension of solubility measurements to yttrium hydroxide (335) indicated this material to be more soluble in ammonium nitrate solutions containing zinc nitrate than in those containing cadmium nitrate. Yttrium was then concentrated in the more strongly basic fractions by ammonia precipitation from 3 *N*

ammonium nitrate solutions containing zinc nitrate along with traces of the cerium earths, gadolinium, dysprosium, and terbium (335).

Experiments upon ammonium nitrate solutions containing lanthanum, praseodymium, neodymium, and samarium nitrates indicated mercuric cyanide to have about the same effect as magnesium nitrate and nickel nitrate to parallel cadmium nitrate but to be less effective (346). The effectiveness of cadmium nitrate for separations in the cerium group was approached by no other material, and a ratio of 3CdO to $1\text{R}_2\text{O}_3$ was recommended (346).

This general procedure was used by Prandtl and Grimm (339) in a futile attempt to locate element 61 in neodymium-samarium fractions and by Prandtl to separate erbium with yttrium and holmium from ytterbium, lutecium, and thulium (336) and to concentrate ytterbium from the least basic fractions of the yttrium earths (337). Wierda and Kremers (430) found the method to be more effective for separating praseodymium from lanthanum than the magnesium oxide procedure but to be less convenient. Wilke-Dörfurt and Schliephake (431) reported better results in the same separation when done in perchlorate solutions containing cadmium ion. Selwood (370) found the ammonia-ammonium nitrate-cadmium nitrate separation to be the best of eight studied for the neodymium-lanthanum separation.

The slow generation of ammonia in solution as the result of the decomposition of certain compounds and the resultant steady increase in pH has interested a number of investigators. Thus, Prandtl and Lösch (341) developed a quantitative method for separating cerium from rare earth mixtures which depended upon the abilities of such cobaltic amines as $[\text{Co}(\text{NH}_3)_5\text{NO}_3](\text{NO}_3)_2$ and, especially, $[\text{Co}(\text{NH}_3)_3(\text{NO}_3)_3]$ not only to oxidize cerous ion but also to liberate sufficient ammonia (because of the weakness with which it coordinates to the cobaltous ion) to precipitate ceric hydroxide without precipitating the more basic trivalent materials. The trinitrato-triammine proved to be the more desirable, since sufficient ammonium ion was generated to prevent completely the precipitation of the trivalent substances. The pentammine, however, is easier to obtain and can be employed if some free acid be added to take care of the excess of liberated ammonia. Cerium can be quantitatively estimated by precipitation in this fashion. Thorium would interfere.

The decomposition of aqueous solutions of hexamethylenetetramine to ammonia and formaldehyde upon boiling is accompanied by a steady increase in pH. Ray (350) found that lanthanum nitrate and chloride solutions gave only faint opalescences upon being boiled with hexamethylenetetramine, while lanthanum sulfate solutions were partially converted to the hydrous hydroxide. Cerous salt solutions gave no precipitates in the cold but were partially precipitated on boiling. With praseodymium and neodymium salt solutions, precipitation was more rapid than with cerium. Yttrium hydroxide was precipitated readily from boiling solutions. Addition of ammonium chloride prevented precipitation, but increasing quantities were required in the series lanthanum, cerium(III), praseodymium, neodymium, yttrium. No separations were reported. Ismail and Harwood (188) found that the weakly basic thorium could be quantitatively separated from both the trivalent rare earth and ceric ions by

the use of 10 per cent hexamethylenetetramine solution at 30°C., if 5 g. of ammonium chloride was present per 100 ml. of solution.

Aqueous solutions of urea decompose upon heating to ammonia and carbon dioxide with slow increases in pH. Selwood (370) found the neodymium content of a lanthanum-neodymium mixture to be altered from 29.6 per cent to 43 per cent in the precipitate and 23 per cent in the filtrate by one urea precipitation but gave no details. Fogg and Hess (140) treated nearly neutral solutions of yttrium earth nitrates containing ammonium sulfate short of precipitation with urea and heated at 90–95°C. for periods of 6–8 hr. The resulting suspensions were filtered, the filtrates treated with more urea, and the heating repeated to produce a series of fractions. The yttrium content increased in the last materials to be precipitated (i.e., the most basic) both in the presence and in the absence of thorium, and the method compared favorably in efficiency with the nitrate fusion, basic nitrite, and chromate methods for the separation of yttrium. These observations have been confirmed by further experiments (230).

5. Organic derivatives of ammonia

Reasoning that ammonia is too strongly basic to effect a rapid separation of the weakly basic erbium earths, Krüss (231) investigated a number of more weakly basic substituted ammonias. Of these aniline proved to be best, and a procedure involving treatment of a neutral chloride solution in 50 per cent ethanol at 90°C. with a 2 per cent solution of aniline in 50 per cent ethanol was worked out. Best results were obtained when only about one-third of the earths present was precipitated, because of the solvent effects of the accumulated aniline hydrochloride (169, 170, 233). The precipitates were granular and easy to filter. Krüss and his coworkers (231, 232, 233, 234) employed the method successfully for the partial separation of small amounts of the erbium and gadolinium earths but found it useless for large amounts of material. Kolb (221) employed aniline in aqueous solution to precipitate thorium and thus separate it from the cerium earths. Baskerville and Stevenson (10) were unable to separate neodymium from cerium earth mixtures by means of aniline.

Extensive investigations of the behavior of lanthanum, ceric, praseodymium, neodymium, zirconium, and thorium salt solutions toward a variety of substituted ammonias have been reported by Jefferson (201) and Hartwell (157). Jefferson found that many aromatic amines would precipitate the tetravalent materials without affecting the trivalent ones but reported no separations of the trivalent materials because of similarities in behavior. Quantitative precipitation of these was obtained only with benzylamine and piperidine. Hartwell extended these observations to many other compounds and listed a number which might be useful for separating the tetravalent elements, but no separations of lanthanum, praseodymium, and neodymium were reported.

Baskerville and Stevenson (10) were unable to separate neodymium from lanthanum with either benzylamine or phenylhydrazine but reported favorable indications with piperidine. Atanasiu (4) showed that pyridine will remove thorium quantitatively from the cerium earth elements.

B. FRACTIONAL DISSOLUTION OF OXIDES OR HYDROUS PRECIPITATES

Treatment of mixed rare earth oxides with dilute acids as a means of dissolving the trivalent materials away from the less basic ceric oxide was first carried out by Mosander (20, 21, 304, 305). This separation has been employed by others (79, 143, 145, 162, 262, 333a, 362, 376a, 454), although the partial dissolution of ceric oxide was early recognized (162, 362). While pure ceric oxide is quite insoluble even in concentrated acids, oxide mixtures containing less than 55 per cent of the material dissolve reasonably readily (79). This suggests a combination between the slightly acidic ceric oxide and the more basic oxides of the trivalent elements. The basicities of adjacent trivalent materials differ so slightly from each other that fractional dissolution of oxides in dilute acids has not proved effective. Marignac (262) effected a partial separation of lanthanum from didymium in this fashion, and others have shown that dilute nitric acid extracts lanthanum, praseodymium, neodymium, and samarium in this order (32).

Sulfur dioxide has been shown to convert the insoluble basic sulfates of praseodymium and neodymium into soluble sulfates more readily than basic ceric sulfate and thus effect a separation (13). Grossmann (153) has found that the ease with which hydroxides are dissolved by aqueous sulfur dioxide increases in the series cerium(IV), didymium, lanthanum.

The solubilities of some of the rare earth oxides in aqueous solutions of ammonium salts have long been recognized (20, 21). Brauner (45) found the relative solubilities of the oxides Di_2O_5 , Di_2O_3 , and La_2O_3 after contact for 24 hr. with a solution containing 31 g. of ammonium nitrate in 600 cc. of water to be as 1 to 10 to 29 and suggested this as a means of separation. Later, he concentrated lanthanum by this method (48, 52). von Scheele (419) found the sesquioxides to dissolve in boiling ammonium nitrate solution and the peroxides to be insoluble; he employed this procedure to remove lanthanum from praseodymium and to separate praseodymium from other materials. Treatment of mixed oxides with boiling ammonium chloride solution was suggested by Watts (426) as a means of dissolving lanthanum and didymium compounds out of ceric materials, but the separation of didymium is not complete (137). The use of boiling ammonium nitrate solution has been suggested for this separation (442).

Fractional dissolution of precipitated rare earth hydroxides in aqueous solutions of aniline hydrochloride at 60°C. was recommended by Krüss (233) as an excellent means of separating the yttrium earths, the course of the separation paralleling that of precipitation with aniline (233). The utility of this method as a means of concentrating the gadolinium, terbium, and holmium earths has been demonstrated (169, 170, 233, 234). Details of the procedure have been excellently summarized by Spencer (379).

A means of separation based upon the oxidation of cerium with chlorine in potassium hydroxide solution and the simultaneous dissolution of the hydroxides of the more basic trivalent elements was proposed by Mosander (304, 305, 306). This method, either as proposed or as modified through the use of other alkalis, has been widely employed for the separation of cerium (47, 67, 71, 100, 101, 136,

156, 159, 202, 213, 313, 331, 333, 436). Browning and Roberts (69) reported better results using bromine instead of chlorine but found iodine to have little solvent effect upon the trivalent materials. Browning (68) reported a greater rate of solution for lanthanum hydroxide than for the didymium compound and effected a better separation of these materials in this fashion than by the conventional double ammonium nitrate procedure.

C. HYDROLYSIS

1. *Separation of cerium by hydrolysis*

Hydrolysis of ceric sulfate or nitrate solutions with the resultant precipitation of basic salts represents an old and often-employed procedure for the separation of cerium from the more basic trivalent materials (32, 252, 379). The method was first proposed by Hermann (162), who separated cerium as a basic nitrate and purified it as a basic sulfate. Lange (238) separated cerium as a basic nitrate after treatment of the ignited oxides with nitric acid. Marignac (262) dissolved the mixed oxides in sulfuric acid and added water to precipitate a basic sulfate. Bunsen (72) obtained better results by pouring a solution containing ceric nitrate into boiling water containing a small amount of sulfuric acid (2 ml. per liter), and it is this modification which has been most widely employed. Many investigators have employed the general method (27a, 47, 49, 50, 51, 54, 56, 70, 73, 99, 123, 124, 136, 137, 145, 156, 162, 175, 277, 284, 290, 333a, 349, 358, 377a, 387, 428a, 434, 437, 440, 443, 444, 447). While the results are excellent, more than a single hydrolysis is necessary for complete purification, and the recovery of cerium is not complete (137). Complete recovery of cerium is possible if the process be made continuous.

Ceric material can be completely precipitated from boiling solutions by sodium acetate (135, 289) and magnesium acetate (289), while the trivalent materials are unaffected. The precipitation of basic ceric nitrate after oxidation with potassium bromate (183, 193, 196), potassium permanganate (30, 138, 191), sodium peroxide (276), and other oxidizing agents (418) represents another application of hydrolysis.

2. *Hydrolysis of nitrites: the "basic nitrite" process*

Although earlier employed by Hofmann and Burger (168) to separate erbium from more basic yttrium earths, fractional precipitation of basic nitrites as an excellent means of removing yttrium from the yttrium earths was first used by Holden and James (172). These authors treated a boiling nitrate solution of the yttrium earths, stirred with live steam, with sufficient concentrated sodium nitrite solution to precipitate about one-third of the rare earth materials present. By reducing this general procedure to a systematic fractionation, it was found possible to recover comparatively pure yttrium from erbium-yttrium and dysprosium-holmium-erbium-yttrium mixtures in relatively few steps, yttrium separating in the more basic fractions.

The fundamental procedure has been retained in many other investigations.

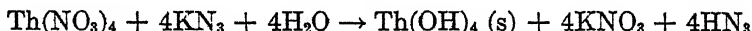
Thus it was employed by James and his coworkers (141, 194, 432) in the recovery of pure yttrium from yttrium-erbium concentrates. Hopkins and his coworkers have employed it in the preparation of yttrium material of atomic weight purity (182), in the removal of yttrium from erbium in the concentration of the latter (38, 429), in the removal of yttrium from holmium (449), in the establishment of the relative basicities of yttrium and the rare earth elements (185, 373, 374), and in the concentration of illinium (178, 179). Thompson, Holton, and Kremers (395) found it advantageous in the removal of the last traces of erbium and holmium from yttrium, and it has been recommended as a good general method for purifying yttrium (226, 371).

The utility of this procedure for the separation of yttrium, particularly from erbium and holmium, is doubtless due to the fact that the basicity of yttrium as determined by this means is greater than that of samarium (185) and thus of any of the yttrium earths.

The work of Sherwood with Hopkins (373, 374) and of Hughes and Hopkins (185) indicates that the basic nitrite procedure will separate all the rare earths in the order of their basicities; hence it should be applicable to many separations besides that of yttrium. A modification of the fractionation scheme to reduce the number of fractions and enhance differences in basicity (374) as well as careful control of the acidity to prevent the formation of colloidal precipitates (374) make the procedure a rapid as well as an excellent one (180). Hopkins (178, 179) has stated that twenty-five basicity fractionations with sodium nitrite effected twice the enrichment of illinium as was accomplished by two thousand double magnesium nitrate crystallizations.

3. *Hydrolysis of azides*

Dennis and Kortright (119, 120) found that the addition of a 0.3 per cent sodium or potassium azide solution to a cold solution containing thorium and rare earth nitrates followed by boiling precipitated thorium quantitatively but not the other materials. The reaction may be represented by the equation (116):



Wyrouboff and Verneuil, however, did not regard the procedure as effective (444).

The effects of alkali azides upon salt solutions of the trivalent materials were first examined by Curtius and Darapsky (98), who found lanthanum nitrate solution to remain clear in the cold and to precipitate a basic azide only on prolonged boiling. Yttrium sulfate solution, however, became cloudy in the cold and deposited a voluminous precipitate on boiling, while cerous and didymium nitrate solutions were intermediate between these extremes. Dennis and Dales (118) attempted to fractionate the yttrium earths by boiling with potassium azide but were unable to effect any separations.

More recently, Komppa and Wuorinen (225) found it impossible to prepare samarium azide from neutral solutions because of hydrolysis and suggested that since samarium was precipitated quantitatively as hydroxide from boiling solutions, azides might be employed for separations. Ant-Wuorinen (2) extended

these observations to a fractionation of the rare earth materials, the azides being hydrolyzed in the order of increasing basicities. A fairly rapid separation was attained by adding the rare earth chloride or nitrate solutions to sodium azide solutions. Cerium was removed from cold solutions by addition of sodium azide and hydrogen peroxide (2), the ceric material hydrolyzing readily and quantitatively.

4. Hydrolysis of miscellaneous compounds

Hydrolysis of phthalate solutions upon warming was recommended by Meyer and Wuorinen (293) as an excellent means of separating yttrium from the erbium earths, the phthalates of the latter elements hydrolyzing the more readily. Yntema and Hopkins (449) used this method for the separation of holmium from yttrium but found it to be far less efficient than the basic nitrite procedure.

Kremers and Balke (227) dissolved mixed holmium and yttrium hydroxides in lactic acid and hydrolyzed the resulting solutions by warming for several hours on a steam bath, but little differences in the compositions of the precipitates and the mother liquors were noted.

Krüss and Loose (235) believed that the more weakly basic elements should yield more readily hydrolyzable salts with chromic acid than the more basic ones. Precipitation of warm neutral solutions with potassium chromate concentrated the yttrium earths in the precipitate and the cerium earths in the filtrate.

By dissolving yttrium group oxalates in ammonium carbonate solutions and boiling to hydrolyze the dissolved double carbonates, James (189) systematically separated erbium from holmium, dysprosium, and terbium.

The separation of calcium from the rare earth elements by preferential hydrolysis of the chlorides of the latter to insoluble basic salts by water vapor at 500–600°C. has been reported (1). Frerichs (142) found basic didymium chloride to be more readily hydrolyzed to the hydroxide than the corresponding lanthanum compound and effected a separation.

Precipitation of basic sulfites from boiling solutions upon treatment with potassium sulfite concentrated yttrium in the most basic fractions (37). Similar results were obtained with sodium citrate, tartrate, tungstate, *m*-nitrobenzoate, and phenoxyacetate and with ammonium camphorate using the same procedure (37).

As a modification of the nitrate fusion procedure (see page 146), Brinton and James (62) treated boiling nitrate solutions of the yttrium earth elements with sufficient sodium hydroxide to form minute crystals of basic nitrates. Upon cooling, the resulting solutions deposited crystalline masses of basic nitrates of the less basic elements. These were removed, redissolved, and retreated as required for systematic fractionation. Rapid separations of erbium and holmium from yttrium were thus effected. Working with chloride or thiosulfate solutions, the authors separated yttrium in a similar fashion. Dissolving basic nitrates in nitrate solutions, followed by evaporation and cooling to deposit more basic nitrates, also permitted separation of yttrium. Fogg and James (141) rapidly removed erbium from yttrium by the same method. This method has

been considered superior to the nitrate fusion because all materials are in solution at the beginning of the process (62).

D. FRACTIONAL PRECIPITATION BY THE ELECTROLYSIS OF AQUEOUS SALT SOLUTIONS

Many years ago, Brauner (45) noted precipitation of didymium material at the cathode when aqueous didymium acetate or sulfate solutions were electrolyzed with platinum electrodes. Since he was interested in anodic products, he did not follow up this observation.

In 1893, Krüss (232) pointed out that upon electrolysis a rare earth chloride solution would evolve hydrogen and chlorine at the electrodes and slowly become more basic. As a result, progressive precipitation of the rare earth elements would occur, the weakest bases precipitating first and the strongest bases last. When a nearly neutral chloride solution of the mixed yttrium earths was electrolyzed at 40°C. with a copper cathode and a carbon anode, a dense granular hydroxide precipitate formed at the cathode. Periodic interruptions of the electrolysis, followed each time by removal of the precipitate, yielded a series of fractions containing materials of steadily increasing basicities. Krüss suggested the method as an excellent one for separations in the yttrium group.

Further work on this general procedure was carried out by Dennis and his students (117, 118, 121, 122, 125, 126, 127). The first experiments by Dennis and Dales (118) upon the electrolysis of a mixed yttrium group nitrate solution with platinum electrodes and at potentials ranging from 2.2 to 2.7 volts yielded white hydroxide precipitates at the cathode, but the differences in average atomic weight among the several fractions were too small to indicate any separations.

Later, Dennis and Lemon (121, 122) reviewed the earlier work and extended the method to separations among the cerium earths. Using a platinum wire as an anode and a mercury surface agitated with a current of air as a cathode, these authors electrolyzed nitrate solutions for varying periods at potentials of about 9 volts. From a neutral solution of the mixed nitrates of lanthanum, praseodymium, neodymium, and samarium, ten hydroxide fractions varying from light brown in color for the first to white for the last were obtained, the final mother liquor yielding no absorption lines and containing only lanthanum nitrate. Electrolysis of a neutral nitrate solution containing lanthanum and praseodymium in roughly equivalent amounts effected rapid precipitation and removal of praseodymium. In like fashion, erbium was rapidly removed from yttrium. Changes in composition were followed by absorption-spectra measurements and atomic-weight determinations. The general procedure used has been covered by a patent (117).

Dennis and van der Meulen (126, 127) electrolyzed both neutral chloride and nitrate solutions in diaphragm cells with stirred mercury cathodes and found separations to be about four times the more rapid from nitrate solutions. A 10 per cent chloride solution prepared from cerium-free oxides of the yttrium group was electrolyzed fractionally, yttrium and neodymium concentrating in the most basic fractions, with holmium, erbium, and thulium in the least basic. Exactly

similar results were reported for a 7 per cent yttrium group nitrate solution, and the separation of even small amounts of neodymium from the yttrium group was accomplished.

Further experiments by Dennis and Ray (125) upon nitrate solutions containing yttrium, holmium, erbium, thulium, and neodymium indicated that the earths were precipitated in the order of their basicities and that the separation increased in efficiency the more vigorously the mercury cathode was stirred. Thorium, when present, concentrated in the first or least basic fractions.

Separation by electrolysis was reinvestigated later by Kremers and his co-workers (228, 229, 312). Neckers and Kremers (312) effected a ready separation of praseodymium from lanthanum by electrolyzing a well-stirred 8 per cent chloride solution in a diaphragm cell at potentials of 6–7 volts with a mercury cathode, 99 per cent lanthanum material being recovered from the final mother liquors. Under similar conditions, pure lanthanum was separated from a mixture of cerium group chlorides containing small amounts of the yttrium subgroup, the less basic earths concentrating in the first fractions. The same separation was accelerated without alteration in efficiency by the addition of 5 per cent sodium chloride. No appreciable separation of praseodymium from neodymium was effected in another electrolysis, and the authors concluded that the separation of lanthanum was the only feasible one in the cerium subgroup.

Kremers and Quill (228, 229), as a result of a comprehensive investigation, proposed the use of cells in which the platinum anode was isolated from the catholyte in a porous cup and in which molybdenum cathodes were employed as being more convenient than those of mercury. Electrolysis of nitrate solutions containing the equivalent of 10 per cent rare earth oxides effected ready removal of small amounts of erbium and holmium from the more basic yttrium. Yttrium was separated from mixtures of yttrium earths containing small amounts of the cerium earths, and neodymium and praseodymium were shown to be definitely more basic than yttrium.

A number of other attempts at electrolytic separations have been made. Thus, Bricout (61) removed cerium by precipitating basic ceric chromate at the anode, lanthanum and didymium being unaffected. Hughes (184) tried unsuccessfully to improve the separation of the yttrium earths by electrolyzing acetate solutions. Selwood (370) altered the amount of neodymium in a lanthanum–neodymium mixture from 29.6 per cent to 60 per cent in one electrolytic precipitation. Yapuncich (448) separated erbium from yttrium by the electrolysis of sulfamate solutions using platinum electrodes, the work being based upon the thesis that sulfamate would undergo anodic oxidation to sulfate and thus give rise to easily filterable basic sulfates. Ant-Wuorinen (3) electrolyzed azide solutions of cerium-free rare earth elements, using platinum electrodes. The weaker bases appeared in the first precipitates, and yttrium concentrated in the middle fractions.

Although it was once believed that ammonia produced by the electrolytic reduction of nitric acid was responsible for cathodic precipitation upon electrolysis (126), this was disproved by the use of chloride solutions (126). It

appears that the process actually depends upon the reduction of water to hydrogen and hydroxyl ion, a change which steadily increases the pH of the solution and slowly precipitates the materials in the order of increasing basicities (126). Since electrolyses proceed at potentials considerably greater than the oxidation potentials which have been measured (126), it cannot be said that separations depend upon electrolyses at potentials intermediate between those characteristic of the elements being separated.

E. HIGH-TEMPERATURE REACTIONS

1. Fractional decomposition of fused nitrates: the "nitrate fusion" process

A favorite method, particularly among early workers, for the separation of the yttrium earths involves melting the mixed nitrates, either alone or in combination with sodium or potassium nitrate, at as low a temperature as possible and then slowly raising the temperature until evolution of nitrogen dioxide fumes begins. Heating is then continued at constant temperature until fumes are no longer evolved, and the mass is then either cooled and treated with only enough water to dissolve the undecomposed normal nitrates or slowly poured into enough water to dissolve it completely, basic salts separating upon cooling (32, 246, 254, 256, 279, 280, 379). Reconversion of the insoluble basic nitrates into normal nitrates, followed by repetition of the decomposition, renders the process fractional in character. In this fashion, the elements can be separated in the order of their basicities (379). The use of acetone as a solvent instead of water has been recommended (8).

Nitrate fusion was first proposed by Berlin (18) and was employed shortly thereafter for separating lanthanum from didymium (100, 101) and for separating erbium from yttrium (91, 92, 113). Chief exponents of the method among early workers were Bahr and Bunsen (6), who systematized Berlin's original procedure; Cleve, who employed the method to separate erbium from yttrium (91, 92) and to separate cerium and thorium from lanthanum (88) but could effect no separation of terbium from yttrium by its use (87, 95); Marignac, who used it to fractionate the yttrium earths (264, 266) and isolate ytterbium (265); Nilson, who isolated scandium by separating it from the more basic ytterbium (316, 318, 320, 322), separated relatively pure ytterbium (317, 319, 321), and removed thorium from rare earth mixtures (236, 237, 323) by means of the procedure; and Urbain, who found that it was not suited to the fractionation of the terbium earths (399) but was suited to the isolation of ytterbium and thorium in the least basic fractions and yttrium in the most basic (405) and to the elimination of most of the yttrium from the earths (403). Auer von Welsbach (422) believed the process to be the best known (at that time) for the separation of large amounts of erbium from yttrium, although he recognized that the last traces of erbium could not be so removed. von Welsbach also used the method to concentrate scandium and ytterbium in fractions less basic than erbium and to separate the cerium earths from the less basic yttrium earths.

In order to lower the temperature necessary for fusion, Debray (112) added

potassium nitrate to the mixed nitrates. Fusion of such a mixture at 300–350°C. effected decomposition of cerium nitrate only, whereas heating above 350°C. was necessary to decompose didymium nitrate and permit a separation from lanthanum. Crookes (93) purified yttrium by nitrate fusion and used Debray's modified procedure to remove cerium (94) and to separate scandium from ytterbium (97). Prior to 1910, nitrate fusion was used by many other workers to fractionate the yttrium earths (22, 23, 40, 81, 169, 212, 234, 367, 369) and the cerium earths (10, 22, 23, 137, 331, 365, 366, 443, 444). Bettendorff (22) obtained clear melts for the yttrium earths because the melting points lay below the decomposition temperatures but always noted decomposition of cerium earth nitrates before fusion. Dennis and Magee (123, 124) were unable to separate cerium from didymium by Debray's method, since didymium nitrate decomposed at 300°C. With a 1:1 potassium nitrate–sodium nitrate mixture (m.p. 231°C.), cerium was always completely freed of didymium. Brauner and Pavliček (58) were more successful in the removal of didymium, and later the modified procedure was applied unsuccessfully to the yttrium subgroup (118).

More recently, the general method has been refined and extended by James and his students (171, 172, 192, 193, 194, 195) and by workers in the University of Illinois laboratories (38, 128, 134, 183, 227, 429, 430). James (192, 193) found that about seventy fusions would free ytterbium from erbium and thulium, but that the complete removal of terbium from yttrium was impossible. James and Pratt (195) showed that the separation of yttrium was accelerated if heating were stopped before the appearance of crystals. The same modification was recommended by James (193) and James and Grant (194), with the further improvement of pouring the fused mass into cold water and collecting the basic nitrate crystals which separated upon cooling. The separation of erbium (193) and holmium (192) from the more basic yttrium was effected, and yttrium–erbium mixtures were systematically fractionated (171, 172).

As a recommended method for the separation of practically pure erbium, nitrate fusion has been employed by Engle and Balke (134), Wichers, Hopkins, and Balke (429), and Boss with Hopkins (38). Of the many methods investigated for this separation, it proved the most desirable (429). Kremers and Balke (227) effected no appreciable separation of yttrium from holmium after twelve fusions of the mixed nitrates. However, thirty-one fusions of a similar mixture after the addition of samarium concentrated yttrium with samarium in the more basic fractions and holmium in the least basic. Driggs with Hopkins (128) found the separation of yttrium from holmium to proceed rapidly until most of the yttrium was removed. Then complete decomposition along the walls of the containers due to local overheating reduced the efficiency of the process. To obviate this, subsequent fusions were effected in a furnace, the temperature of which could be controlled accurately, and holmium of atomic weight purity was separated after one hundred ninety fusions. The same furnace was used effectively in the preparation of pure erbium (38) and in a preliminary separation of praseodymium from lanthanum (430).

As a method of concentrating scandium and ytterbium, removing yttrium

from the yttrium earths, and removing cerium, nitrate fusion can be recommended, although accurate temperature control and uniform heating are necessary.

2. Fractional decomposition of sulfates

Although Hofmann and Burger (168) employed fractional thermal decomposition of the sulfates of the yttrium earths as a means of separation, their data are incomplete.

As has already been pointed out, Wöhler and Grünzweig (439) measured the sulfur trioxide pressures above a number of sulfates with the object of establishing conditions under which one sulfate might decompose while the others remained stable. Appreciable temperature differences at the same sulfur trioxide pressure were found only between cerium(III) and scandium, between scandium and samarium, and between yttrium and lanthanum. Effective separations of scandium and possibly yttrium from lanthanum appeared to be the only feasible ones. When neodymium and praseodymium sulfates were heated together at 1060°C. (pressure above the neodymium compound 1520 mm. and that above the praseodymium compound 1250 mm.) and the cooled product leached with water, no separation was noted. Wöhler and Flick (438) listed definite differences among the dissociation temperatures of the sulfates of lanthanum, praseodymium, neodymium, and cerous and ceric cerium, but the differences were not excessively large and no separations were attempted.

Using a special furnace the temperature of which could be controlled within 0.1°C., Willard and Fowler (433) found that because of isomorphism and resultant formation of solid solutions, lanthanum, cerous, praseodymium, and neodymium sulfates did not exert their own individual dissociation pressures in mixtures, the true dissociation pressures in such mixtures lying between those for the components. If the oxides produced were also isomorphous, e.g., for lanthanum and neodymium, separation by controlled heating was impossible. With cerous sulfate and the others, separations were possible because the resultant ceric oxide is not isomorphous with the oxides of the trivalent materials. For the same reason, i.e., production of a higher oxide, some separation of praseodymium from lanthanum was effected.

3. Miscellaneous thermal decomposition procedures

Pattison and Clarke (329), by heating the mixed chromates of lanthanum, cerium, and didymium to about 230°F., effected a complete decomposition of the cerium material, the others remaining as water-soluble chromates. Removal of cerium was said to be quantitative. Dennis and Dales (118) were unable to separate yttrium from the yttrium earths by a modification of this procedure.

Although Cleve (85) fractionally decomposed hydrated chlorides in investigating didymium chemistry, most of the information available on this process is due to Gibbs (146, 147). Gibbs dissolved mixed oxides in hydrochloric acid, evaporated to a thick syrup, heated for a time in a muffle furnace, and dissolved out the undecomposed materials with water, the undissolved material being re-

processed. A fairly rapid concentration of yttrium in the more basic fractions was achieved, and the method compared favorably with nitrate fusion. Similar experiments on basic bromide formation were inconclusive (147). Strictly speaking, basic chloride and bromide formation represent hydrolytic processes rather than thermal decompositions, but they are better compared with other thermal processes than with the usual hydrolysis procedures.

F. SUMMARY

Consideration of the results achieved with basicity separations leads one to conclude that they can be effective only if sizable differences in basicity exist between the materials to be separated. The removal of lanthanum, yttrium, ytterbium, scandium, ceric cerium, and thorium are cases in point. In combination with other separational procedures, however, they can be extremely useful.

In spite of the abundant use of basicity methods by many workers, most of the procedures outlined are still highly empirical in character. This is, of course, understandable in view of the scarcity of many of the elements in question and the unavailability of pure materials for use in independent studies. Where such pure or comparatively pure materials have been available, say with the cerium earths, much less confusion exists, and the procedures are in general better understood and more highly systematized. It seems safe to predict that as the less familiar materials become better known and more available, many of the apparent anomalies now existent will disappear. Comprehensive physicochemical investigations of many of the procedures are indicated.

Basicity separations, like other fractional procedures with these elements, have suffered because the lack of rapid and accurate analytical procedures has prevented the exact establishment of the courses of fractionations and their efficiencies. As a consequence, systematic procedures have been adhered to very exactly. While this is sound practice, accurate knowledge of the compositions of the various fractions might well suggest combinations and short-cuts not apparent in such systematic schemes.

IV. CONCLUSION

While important deviations are apparent between the theoretically predicted and experimentally ascertained basicities as well as among the results of various separational procedures, these are offset in large measure by the agreements. When considered from a broad point of view, basicity characteristics among the elements of this periodic family represent excellent verifications of trends based upon purely theoretical approaches and are thus of no little importance. Within the rare earth group itself, observed trends are in almost exact agreement with those predicted from the increased attraction for electrons produced by the lanthanide contraction and are thus of importance in elucidating not only the properties of the rare earth elements but also those of succeeding elements in the periodic classification. Many apparent anomalies in properties among all these materials are rendered logical and apparent when this contraction effect is correctly considered.

All lines of evidence support a decrease in basicity in the rare earth series from lanthanum regularly to lutecium, with scandium and ceric cerium following lutecium in order. The position of yttrium as ascertained by measurements upon pure yttrium compounds is in the neighborhood of holmium in the rare earth series, but measurements upon natural mixtures ascribe a somewhat higher basicity to this element.

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CHEMICAL CHARACTERISTICS AND PHYSIOLOGICAL RÔLES OF GLUTAMINE

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CONTENTS

I. Chemical characteristics of glutamine.....	162
A. Properties and reactions of glutamine....	162
B. Methods for the determination of glutamine.....	167
1. Methods involving measurement of ammonia.....	167
(a) Non-enzymatic hydrolysis.....	167
(b) Enzymatic methods—glutaminase....	168
2. Chloramine T and succinoxidase	168
3. Methods involving measure of decreased reactivity on ring closure.....	168
(a) Nitrous acid.....	168
(b) Ninhydrin.....	169
C. Presence of glutamine in the protein molecule.....	169
D. Catalytic hydrolysis of amide groups in proteins by anionic detergents.....	172
II. The rôle of glutamine in plants.....	174
A. The higher plants.....	174
1. Origin of nitrogen of amides....	174
2. Origin of the carbon skeleton of amides.....	176
3. Amide metabolism in detached leaves.....	179
4. Presence of amide hydrolase in plants.....	180
B. The lower plants.....	182
1. Glutamine in the physiology of bacteria.....	182
2. Yeast.....	184
III. The rôle of glutamine in animal metabolism.....	184
A. Presence of glutamine in blood.....	185
B. Presence of glutamine in cerebrospinal fluid.....	186
C. Presence of glutamine in joint fluid.....	186
D. Presence of glutamine in urine.....	186
E. Presence of glutamine in egg.....	186
F. Rôle of the glutamine—glutamic acid—glutaminase system in the central nervous system.....	187
G. Rôle of glutamine and glutamic acid in muscle metabolism.....	188
H. Rôle of glutamine in liver.....	189
1. Deamination-transamination.....	189
I. Rôle of glutamine in urea formation.....	190
1. Krebs's mechanism.....	190
2. Glutamine mechanism.....	191
3. Consideration of energy relationships in urea synthesis.....	191
4. An evaluation of criticisms of Krebs's theory.....	192
J. Rôle of glutamine in detoxification.....	195
K. Rôle of glutamine in the kidney.....	195
1. Glutamine as precursor of urinary ammonia.....	195
2. Presence of glutaminase in human kidney.....	197
3. Glutamine and citric acid metabolism.....	198

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L. Rôle of glutamine in miscellaneous systems.....	198
1. Relation of glutamine to reamination of nucleotides or nucleosides.....	198
2. Presence of free glutamine in animal tissues.....	199
3. Effect of glutamine on carbohydrate metabolism.....	200
4. Effect of glutamine on purine metabolism.....	200
M. Physiological rôles of glutamine in mammals.....	200

Plant physiologists for the past seventy-five years have attached considerable importance to their observations that the amides of the dicarboxylic amino acids were present in considerable concentration in plant tissue in free form, and postulated that the amides were present also, combined with other amino acids, in the protein molecule. Nevertheless, there grew a tendency among many protein chemists and physiologists to focus attention on the more stable amino acid residues in hydrolysates. This became increasingly true after 1873 when Hlasiwetz and Habermann (97) introduced the use of hydrochloric acid with stannous chloride in place of sulfuric acid, and thereby made acid hydrolysis more popular as a tool for the study of the units of protein structure. Labile amides destroyed by hydrolysis with strong acids were absent from the hydrolysates under investigation and for this reason received decreasing attention. While it was still commonly acknowledged that the ammonia liberated by the hydrolysis of protein probably arose from labile amide groups, it has been only recently that the rôle of these amides again began to receive due consideration.

The biological significance of amides has been reviewed extensively in Russian (67), but no comprehensive treatment of the rôle of glutamine has been available in English. The recent crescendo of interest in the rôle of glutamine (6, 7, 57, 58, 68, 69, 85, 86, 87, 89, 153, 268), in animal physiology especially, makes timely both a review of the literature on the subject and an attempt to correlate observations in the light of present knowledge.

I. CHEMICAL CHARACTERISTICS OF GLUTAMINE

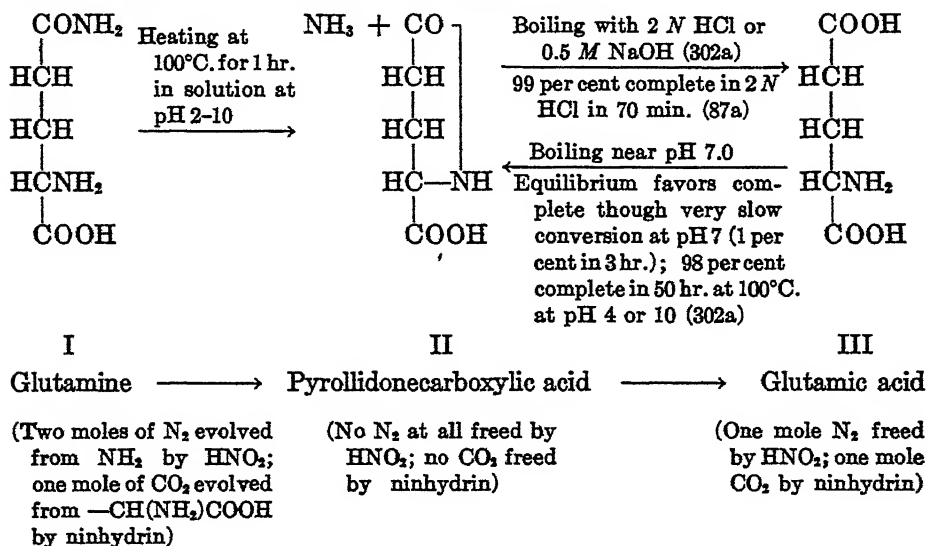
So that the rôle of glutamine in metabolic processes may be better understood, let glutamine be considered first as a chemical substance, apart from its physiological rôles. As an amide of glutamic acid it is a neutral reservoir and precursor of ammonia, glutamic acid, or α -ketoglutaric acid. Obviously, consideration of the rôle of glutamine will involve simultaneous regard for the rôles of glutamic acid and ammonia as well as of the closely related asparagine. For this reason considerable attention will be devoted in the following review to the function of glutamic acid and asparagine.

A. PROPERTIES AND REACTIONS OF GLUTAMINE

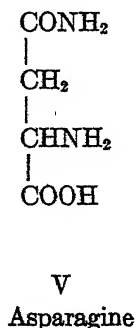
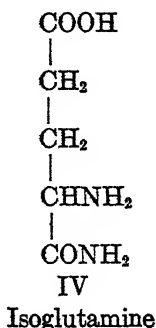
Glutamine has a molecular weight of 146.15 and an elementary composition of C = 41.09 per cent, H = 6.90 per cent, O = 32.84 per cent, and N = 19.17 per cent. Isoglutamine and ammonium pyrrolidonecarboxylate have the same molecular weight and elementary composition as glutamine. Glutamine is soluble in water (3.6 parts in 100 of water at 18°C.) but only very slightly soluble in absolute methyl alcohol (about 3.5 mg. per 100 cc. at 25°C. (87a))

or ethyl alcohol (0.46 mg. per 100 cc. at 23°C.) and almost insoluble in ethyl acetate, chloroform, ethyl ether, and acetone.

Bergmann *et al.* (15) give the melting point of synthesized and natural (beet) glutamine as 184–185°C. McIlwain *et al.* (153) report that glutamine isolated from horse meat melts at 205°C. The melting point of ammonium pyrrolidone-carboxylate is 185–186°C. (9,138). It is of interest in this connection that glutamine (from beets), when heated under the conditions of melting-point determination to 180°C. for 2 min., undergoes less than 5 per cent decomposition, whereas glutamine, similarly heated to 186°C. for 30 sec. (until it is all melted), is quantitatively converted to ammonium pyrrolidonecarboxylate (9). Glutamine is known to be capable of ring condensation to form pyrrolidonecarboxylic acid, while severe acid hydrolysis yields glutamic acid. A quantitative study of these reactions showed that they could be used both to characterize and to determine glutamine in solution. The reactions are:

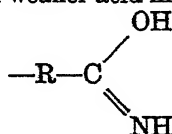


For purposes of comparison, the formulas of isoglutamine (IV), which is an isomer of glutamine (I) and of pyrrolidonecarboxylic acid (II), and of the homologous amide, asparagine (V), are given below.



The nitrogen evolved by reaction with the amino groups of I and III can be determined by Van Slyke's nitrous acid method for amino nitrogen, and the carbon dioxide from amino acid carboxyl groups of I and III by the ninhydrin reaction recently published from this laboratory (85, 86). Glutamine is believed to be the only substance known which shows the peculiar decrease in amino nitrogen and "carboxyl" (87) carbon dioxide on heating at pH 2, and the subsequent increase on boiling with hydrochloric acid. The decrease in carboxyl carbon dioxide caused by heating at pH 2 can be used for quantitative estimation of the glutamine.

Free glutamine is unique as an amide. As suggested by Chibnall (46), it might be considered a hybrid between a true amide and ammonium glutamate. The amide group is much more labile than that of other common amides. Glutamine reacts with nitrous acid in acetic acid, giving nitrogen equivalent to 180 per cent of the α -amino nitrogen (50, 111, 232, 261, 280), whereas asparagine yields nitrogen equivalent to only 100 per cent of the α -amino nitrogen, and amides in which there is no amino group in the α -position to a carboxyl group yield no nitrogen (265). Indeed Schulze and Bosshard (232), who first isolated glutamine, had shown in 1883 that the nitrous acid method of Sachsse and Kormann (211) ($\text{KNO}_2 + \text{HCl}$), a forerunner of Van Slyke's method (265), gave nearly double the expected yield of nitrogen when applied to glutamine. As pointed out by Plimmer (181), the nitrogen of any amide reacts completely with nitrous acid in the presence of a strong acid such as 2*N* hydrochloric acid. This suggests that in solutions of weaker acid most amides exist as the enol form:



This theory is used by Chibnall and Westall (50) to explain the unique behavior of glutamine in nitrous acid. They assume that glutamine in acetic acid exists

as RCNH_2 . Lichtenstein (140) observes that α -alkylamides of glutamic acid also react in the Van Slyke amino method to give 90 per cent of their total nitrogen, and argues that since no primary amido group is present in the alkylamides, the explanation of Chibnall and Westall cannot hold. Lichtenstein suggests that the unique behavior of glutamine is due to preliminary reaction of nitrous acid with the α -amino group, followed by lactone closure and reaction with the amide group as it is liberated. This explanation would stand only on the assumption that nitrous acid will react to the extent of 80 per cent with amide nitrogen *at the moment* it is being hydrolyzed. Once ammonia is formed, nitrogen equivalent to only 25 per cent is formed under the conditions of the analysis (265).

Thierfelder *et al.* (261) showed in 1919 that di- and tri-peptides of glutamine, acetyl derivatives, and gliadin split off ammonia much less readily than did glutamine in 1 *N* sulfuric acid at 20° and 100°C. These findings were confirmed

by Chibnall and Westall (50), using Thierfelder's preparation of leucylglutamine. Nevertheless, Melville (148) reports that in the glutamine peptides which he synthesized the amide group was very labile.

Chibnall (46) is of the opinion that once glutamine is combined with other amino acid residues, as is the case in a protein, its amide group becomes more stable and more closely simulates in properties other amides.

The similarity of structure of amides and peptides is noteworthy. Peptides are indeed substituted amides (in most cases monosubstituted amides). That one carboxyl group of glutamic (or aspartic) acid should form a "peptide" with ammonia is not surprising, in view of this similarity of structure. The similarity of the peptide and amide bonds is indicated also by the similar behavior of the two bonds when subjected to hydrolysis catalyzed by sulfate or sulfonate groups. This type of hydrolysis will be discussed below under the heading "Presence of glutamine in the protein molecule" (see page 169).

It is worthy of note that several investigators, in their attempt to develop methods of analysis for glutamine, have been unable to obtain theoretical yields when they analyzed what they believed to be pure glutamine. The yields have ranged from 80 to 95 per cent of the expected values, suggesting either that glutamine was participating in some unrecognized side reaction or that it existed, in part, in some form the properties of which are not indicated by the recognized structural formula which Thierfelder (258) showed was consistent with most of the properties of glutamine.

Thus Cohen (51), analyzing for glutamic acid by conversion to succinic acid and subsequent use of succinoxidase, obtained almost theoretical yields (94 per cent) with glutamic acid but only 70 to 80 per cent of the expected succinic acid yield with glutamine. The yield was not increased by preliminary hydrolysis of glutamine with acid or alkali. These observations were confirmed by Örström *et al.* (170).

Assuming that in the nitrous acid method of Van Slyke (265) reaction with the α -amino nitrogen of glutamine is complete, only 84 per cent of the amide nitrogen is liberated (50) in 10 min. at room temperature and 95 per cent after 2.5 hr. Vickery *et al.* (280) report that in 4 min. at 22.5°C. 90 per cent of the total nitrogen (80 per cent of the amide) is liberated in the nitrous acid reaction.

Failure of other methods to give theoretical yields with glutamine is due probably to the use of impure preparations, or, in the case of some methods involving hydrolysis, to incomplete reaction under the conditions employed.

Glutamine when heated in weak acid, neutral, or alkaline solution is converted to pyrrolidonecarboxylic acid or its salt much more rapidly than is glutamic acid. At pH 6.5, 99 per cent of the glutamine is converted in 1.5 hr. at 100°C. to pyrrolidonecarboxylate, and the other 1 per cent is converted to ammonium glutamate (86). The main product obtained by non-enzymatic decomposition of glutamine differs from that (ammonium glutamate) obtained by enzymatic hydrolysis, as shown by Krebs (112) and Leuthardt (131) using the nitrous acid technique and, as confirmed in this laboratory, by use of the ninhydrin manometric method (10). The effect of pH and temperature on the rate of

conversion of pyrrolidonecarboxylic acid to glutamic acid (and *vice versa*), as well as the composition of the equilibrium mixtures, have been studied by Wilson and Cannan (302a), Vickery *et al.* (280), Olcott (168a), and Hamilton (87a).

Lichtenstein's (138, 139) claim for conversion of ammonium pyrrolidonecarboxylate to glutamine was later (140) rescinded. He states, however, that alkylamines in aqueous solution react with pyrrolidonecarboxylic acid to yield α -alkylamides of glutamic acid. Besides yielding 90 per cent of total nitrogen in the Van Slyke apparatus, these products were stated to give a strong ninhydrin (colorimetric?) reaction.

It was of interest to note that the relatively stable amide, asparagine, was known sixty-three years before Ritthausen (203) in 1869 isolated the corresponding free acid by acid hydrolysis of conglutin. That same year he postulated the presence of the amide in protein. Glutamic acid, however, was isolated by Ritthausen (202) in 1866 from gliadin. Not until seven years later (97) was the suggestion made that the corresponding amide occurred in protein, and it was seventeen years later before this labile amide was recognized and isolated by Schulze and Bosshard (232) from beet juice.

The structural similarity of pyrrolidonecarboxylic acid to proline makes the observed biological conversion of proline to glutamic acid and glutamine in kidney and perhaps also in liver (164) less surprising. Weil-Malherbe, and Krebs (302) noted that guinea pig and rabbit kidney oxidized proline (although not pyrrolidonecarboxylic acid) to glutamic acid. When ammonium salts were present glutamine was a product. Hydroxyproline gave rise to a small amount of a glutamine-like substance. Krebs (115) showed that proline and ornithine give the same oxidation product after treatment with *D*-amino acid oxidase and are converted to glutamic acid *in vivo*. Roloff, Ratner, and Schoenheimer (206), after feeding deuterioornithine to mice, recovered deuteroproline and deuteroglutamic acid (*cf.* also 236). Borsook and Dubnoff (22) indicate that ornithine is rapidly converted to glutamic acid in rat kidney. Pedersen and Lewis (174) have observed rapid formation of urea after feeding either glutamic acid or proline and quote the previous findings of other authors, supposedly to imply that they feel that proline is converted to glutamic acid.

The similarity in structure of citrulline and glutamine has been discussed (7); in this connection it is interesting to note that Wada (293) claims that boiling citrulline in concentrated hydrochloric acid for 8 hr. gives rise to proline.

Although at least 95 per cent of the glutamine obtained by the synthetic method of Bergmann, Zervas, and Salzmänn (15) is the isomer corresponding to the glutamic acid used as starting material (9), nearly all the glutamine available commercially in this country has so far been prepared from beets by the method of Vickery, Pucher, and Clark (278). Owing to the recent heavy demands for glutamine, a number of commercial houses have undertaken to prepare the material. Some, not appreciating the instability of this amide or misled (140) by early papers by Lichtenstein (138, 139), have offered as glutamine a product over 95 per cent of which is ammonium pyrrolidonecarboxylate. Because glutamine and its decomposition product, ammonium pyrrolidonecarboxy-

late, have identical empirical formulas, elementary analysis gives no clue as to the purity of such preparations. Heretofore there has been published no simple procedure for testing roughly the purity of glutamine preparations, or the concentration of its most common contaminant, ammonium pyrrolidone-carboxylate. Gradual formation of color with Nessler's reagent was observed by Schlenker (219). If to a solution of several milligrams of a preparation in 2 cc. of water one adds 0.5 cc. of the Nessler reagent, one may quickly obtain information as to the quality of the glutamine (9). A good preparation (i.e., one in which there is little or no preformed ammonia) gives no immediate color, but because of the alkalinity of the Nessler reagent glutamine amide nitrogen will split off as ammonia at the rate of about 1 per cent every 5 min. (6). If ammonium pyrrolidonecarboxylate alone is present, maximum color will be obtained immediately on addition of the Nessler reagent. Accurate methods of analysis are included among the following.

B. METHODS FOR THE DETERMINATION OF GLUTAMINE

The methods of glutamine assay available for study of the rôle of this amide can be reviewed briefly. With the exception of Cohen's method, all of these methods involve hydrolysis of the amide linkage. One group of methods depends on the determination of the ammonia liberated by mild hydrolysis with dilute hydrochloric, sulfuric, or trichloroacetic acid, or with alkali, or with heat at neutral pH, or by the action of the specific enzyme glutaminase. Other methods measure the decreased reactivity with nitrous acid or ninhydrin that occurs when glutamine undergoes hydrolysis and condensation to ammonium pyrrolidonecarboxylate.

1. *Methods involving measurement of ammonia*

(a) *Non-enzymatic hydrolysis:* The oldest method involves measurement of ammonia after mild acid hydrolysis with hydrochloric or sulfuric acid and is dependent on the greater lability of the amide group of glutamine as compared with that of asparagine. Chibnall and Westall (50) and Vickery *et al.* (280) have worked out this method in greatest detail. Krebs (112) and Ferdman *et al.* (69) used ammonia production on 5-min. hydrolysis at 100°C. with 5 per cent sulfuric acid as an index of glutamine. Krebs pointed out that this procedure, besides liberating all the glutamine amide nitrogen, liberated 23.2 per cent of the amide nitrogen of asparagine. Örström *et al.* (170) had used the same hydrolysis for 10 min. Schlenker (219) reviewed methods of glutamine determination up to 1932. After removing preformed ammonia with sodium permittit (218, 219), he heated plant extracts for 2 hr. at 100°C. in 0.2 *M* phosphate buffer (pH 6.0–6.5) to distinguish between glutamine and asparagine. Wood *et al.* (303, 304, 305) used similar conditions. No correction for ammonia liberated from urea was made by any of these workers, although Chibnall and Westall (50) noted that 22.9 per cent of urea was hydrolyzed in 1 *N* sulfuric acid in 1 hr. at 100°C. Harris (89) has measured the *rate* of ammonia liberation on hydrolysis with 10 per cent trichloroacetic acid at 50° to 80°C., and has used

this as a qualitative test for, and as a measure of, glutamine amide nitrogen in plasma and spinal fluid. After $1\frac{1}{2}$ hr. at 70°C . glutamine was completely hydrolyzed, whereas only 12–15 per cent of added asparagine was hydrolyzed. Harris recognized the importance of correcting for the ammonia split off from urea during acid hydrolysis and selected his conditions to minimize urea breakdown. Steinhardt and Fugitt (248), by the use of catalysts such as dodecyl sulfate, have extended the acid hydrolysis method to measure glutamine amide nitrogen as well as of asparagine amide nitrogen in proteins. A slight modification of this method and the use of commercially available detergents have provided the author (9) with similar data on other proteins.

Efimenko and Naugolnaya (61) used alkali (sodium carbonate) to decompose amines and amides, but this method is less specific. Possible decomposition of arginine, citrulline, and canavanine would have to be considered. Mendel and Vickery (149) determined glutamine by hydrolysis at 100°C . in a buffer at pH 6.5. Mothes (159) and Schlenker (219) used similar conditions. Chibnall and Westall (50) observed that glutamine was almost completely hydrolyzed after 3 hr. at 100°C . and pH 8. Asparagine, however, was very little affected by such conditions.

(b) *Enzymatic methods—glutaminase*: The author (5, 6, 7) has found the measurement of ammonia formed by glutaminase action most convenient for measuring glutamine in dog and human blood plasma. This procedure has the advantage over methods involving acid hydrolysis in that it is more specific in the presence of urea and asparagine; the method is one of promise for the determination of free glutamine in enzymatic hydrolysates of proteins (9, 55). The weakness of the method rests on the fact that the glutaminase preparations liberate ammonia from the adenosine and its derivatives present in tissues. It is probable, however, that the glutamine amide nitrogen of tissues can be determined enzymatically as the difference between the ammonia nitrogen liberated after digestion with the glutaminase preparation and that liberated after digestion with the glutaminase preparation + bromsulfalein. Bromsulfalein inhibits glutaminase (7) but not adenosine deaminase (9).

2. Chloramine T and succinoxidase

Cohen (51) oxidized glutamine or glutamic acid to succinic acid and then measured the latter, using succinoxidase. This method, however, does not distinguish between glutamic acid and its amide.

3. Methods involving measure of decreased reactivity on ring closure

(a) *Nitrous acid*: When glutamine is heated at a pH near 7 it is converted rapidly to ammonium pyrrolidonecarboxylate. The nitrogen in the ring of this product reacts inappreciably in the Van Slyke nitrous acid method for amino nitrogen and only 25 per cent of the nitrogen in the ammonium radical reacts. When the pyrrolidonecarboxylate is heated with strong acid, the ring is hydrolyzed with the formation of glutamic acid.

The decrease in the nitrous acid amino value, resulting from heating glutamine at a pH near 7 (280), and partial restoration of this value following hydrolysis of pyrrolidonecarboxylate to glutamic acid by heating (100°C. for 2 hr.) in 2 *N* hydrochloric acid, have been used by Pucher and Vickery (192) as an index of glutamine content. Pucher and Vickery separated the pyrrolidonecarboxylic acid from ammonia and urea by extracting the acid with ethyl acetate.

(b) *Ninhydrin*: Glutamine reacts with ninhydrin, liberating carbon dioxide equivalent to the α -amino nitrogen. Pyrrolidonecarboxylic acid does not react. Neuberger and Sanger (165) measured the carbon dioxide produced in the ninhydrin reaction before and after heating for 3 hr. at pH 6.8 and considered the drop in the carbon dioxide production as a measure of glutamine. This method was developed independently (85, 268) and worked out in detail by Hamilton (86). Hamilton has drawn attention to the instability of glutamine in the presence of such anions as phosphate, and has made his method specific for glutamine in blood and tissues by removing such interfering substances as ascorbic acid and glutathione by preliminary precipitation at pH 6.5 with neutral lead acetate. Under the conditions employed by Hamilton, less than 0.1 per cent of glutamic acid is converted to pyrrolidonecarboxylic acid and less than 0.1 per cent of asparagine is hydrolyzed. The fact that asparagine does not interfere gives the ninhydrin method an advantage over the glutamine methods, which measure ammonia produced on acid hydrolysis. This advantage is of particular importance in dealing with plant tissues. The fact that adenosine and its derivatives do not interfere gives the method an advantage over the glutaminase method and permits measurement of glutamine in animal tissues.

C. PRESENCE OF GLUTAMINE IN THE PROTEIN MOLECULE

Ritthausen in 1869 (204, 205) recognized a product of acid hydrolysis of conglutin (from lupin seeds) to be the same (aspartic acid) as that given by hydrolysis of asparagine and postulated that asparagine was probably present in the protein molecule. Nasse (161, 162, 163) in the same year concluded that the ammonia produced on acid hydrolysis of proteins came from acid amide groups like that in asparagine.

Hlasiwetz and Habermann in 1873 (97), from experiments on acid hydrolysis, concluded that casein was composed of units including aspartic acid and asparagine, glutamic acid, and glutamic acid amide. Their suggestion that the protein contained glutamine preceded by ten years the discovery of glutamine by Schulze and Bosshard (232).

In 1877 Schulze (223) postulated that since glutamic acid could be separated from beet juice and pumpkin seedlings (231) only after an acid hydrolysis which was accompanied by formation of ammonium ion, glutamic acid existed in beets (or pumpkin seedlings) in the form of glutamine. He suggested later that glutamine, which he found free in germinating plants, was derived from the protein, and that after germination the glutamine was reconverted to protein. In 1883 Schulze and Bosshard (232) isolated glutamine from beets.

In 1904 Emil Fischer (72) and in 1908 Osborne, Leavenworth, and Brautlecht

(171, 172) agreed that most of the ammonia produced on acid hydrolysis of proteins came from combined glutamine and asparagine. Indeed, Osborne *et al.* stated that the ammonia production observed was equivalent, in the case of most proteins, to the sum of the aspartic and glutamic acids which could be isolated from the hydrolysates. They concluded that these amino acids in the protein molecule exist as peptides of asparagine and glutamine. They noted (172) that on boiling a solution of gliadin in 20 per cent hydrochloric acid ammonia production was complete in 30 min., as was the case also with asparagine. Since the amounts of dicarboxylic amino acid isolated were somewhat smaller than the amounts present in the proteins or their hydrolysates, it might be concluded that a small part of these dicarboxylic acids existed as such rather than as amide in the peptide chain. This would seem to be especially true in the case of three pea proteins studied by Osborne *et al.* In the case of cereal proteins, however, the yield of ammonia was so much more than the amount equivalent to the sum of the two dicarboxylic acids isolated that Osborne *et al.* postulated the existence of a third dicarboxylic amino acid in the protein molecule. The contention that amides existed in the protein molecule was supported in 1912 by Van Slyke (266), who showed also that the ammonia produced was greater than could be expected from cystine, which was the only non-amide amino acid giving appreciable ammonia on acid hydrolysis at 100°C. (see also 168a). Thierfelder *et al.* (261) in 1919 prepared some glutamine peptides and postulated their occurrence in proteins. Actual proof that the ammonia produced on acid hydrolysis arose from amides of amino acid came in 1932, when Damodaran (54) isolated asparagine from edestin and later with Jaaback and Chibnall (55) obtained glutamine from the enzymatic digest of gliadin. In 1935 Shore (242) showed that on acid hydrolysis of protein there was first a rapid rise in ammonia production, followed by a slower rise and humin formation. He calculated that on the assumption of a molecular weight of 34,500 for egg albumin, twenty-four amide groups per mole were present.

In 1935 Melville (148) synthesized glutaminyl peptides and postulated their natural occurrence. The next year the presence of glutamine in insulin was suspected by Harington and Mead (88).

Wormell and Kaye (307) report that animal and plant caseins are deamidated by the action of 1 per cent sodium hydroxide for 40 hr. at 45°C. No data are given to indicate whether this technique splits off all amide groups or whether it causes any liberation of ammonia from combined arginine or citrulline. They claim that in neutral solutions (near the isoelectric point of protein) formaldehyde combines with the free amino groups of protein (especially of lysine). In acid solution formaldehyde combines also with amide groups. In view of this finding it is pertinent to enquire whether or not formaldehyde was used by Miles and Pirie during the preparation of the antigen of *B. melitensis*. As noted below, Philpot (177) reported that formic acid was split off from this antigen by the action of detergents.

Fraenkel-Conrat *et al.* (73a) likewise conclude that formaldehyde combines with the primary amino groups and primary amide groups of proteins and poly-

peptides but not with the secondary amide linkages of the peptide chain. This conclusion was reached partly by comparing the amount of formaldehyde bound by (a) polyglutamic acid synthesized by *B. subtilis* and by (b) polyglutamine synthesized *in vitro* from polyglutamic acid methyl ester.

Synge (254) subjected glutamine in the protein molecule (gliadin) to Hofmann degradation by treating with cold hypobromite, heating to 80°C., then hydrolyzing the peptide bonds by refluxing with hydrochloric acid. He obtained α,γ -diaminobutyric acid equivalent to 16.5 per cent of the glutamic acid residue and concluded that at least this much glutamic acid existed as the amide in the protein.

Chibnall (47, 48) in 1942 listed the amide nitrogen content of thirteen proteins. Gliadin has 25.78 per cent of its total nitrogen in the form of amide nitrogen and corresponding figures for other proteins are zein 18.3 per cent; edestin 9.49 per cent; casein 9.05 per cent; gelatin 0.5 per cent. By reasoning on the grounds of a revised Bergmann-Niemann hypothesis, Chibnall concludes from the total ammonia liberated on acid hydrolysis and the number of glutamine and aspartic residues, that only a portion of the dicarboxylic acids exist as the amides. In the case of edestin, for every unit containing 432 amino acid residues, 72 were glutamic acid, of which 36 were believed to be present in the protein as a peptide of glutamine, and 45 were aspartic acid, of which 27 were believed to preëxist as peptides of asparagine. From the amount of ammonia liberated on acid hydrolysis it was concluded that in β -lactalbumin only 32 of the 93 dicarboxylic acid residues could exist as amide in the protein, whereas for egg albumin only 31 of 75 dicarboxylic acid units were amides. Clearly, then, the statement by Everett (65) that "Distillation of protein hydrolysates with magnesium oxide *in vacuo* and determination of the ammonia in the distillate provides a quantitative measure of the dicarboxylic content of proteins" can be true only in so far as the result indicates, in some cases, a minimal value for dicarboxylic acid, and in other cases includes some of the nitrogen of citrulline.

Brand *et al.* (29a), in the most complete analysis of a protein yet recorded, account for 99.3 to 99.6 per cent of the amino acid present. They state that a mole of crystalline β -lactoglobulin (mol. wt. 42,020), found in whey of cow's milk, contains 36 moles of aspartic acid and 56 moles of glutamic acid. The amide nitrogen found was adequate to account for only 32 (of the 92) dicarboxylic acid residues existing preformed as amides in the protein molecule. Hence in this protein (unlike those described by Osborne *et al.*), most of the dicarboxylic acid residues are not present as amides.

Bergmann and Fruton (14) have considered the possibility that glutamine reacts with keto acids to form CO—NH— bonds involving the γ -carboxyl of glutamic acid, similar to a bond already recognized in glutathione.

Protein denaturation by detergents has been discussed in a review (166) by Neurath *et al.* The action on proteins of cationic detergents such as aryl alkyl substituted ammonium halides has been described by Kuhn *et al.* (125) and Schmidt (220). The effect of only the anionic detergents will be considered here.

D. CATALYTIC HYDROLYSIS OF AMIDE GROUPS IN PROTEINS BY ANIONIC DETERGENTS

Steinhardt and Fugitt (248) provided a simple technique for measuring the amount of glutamine (also of asparagine) present in proteins by plotting, against time, the amount of ammonia liberated from a protein when heated to 65°C. with 0.05 *M* hydrochloric acid and 0.03 *M* neutral salt of a catalyst such as dodecyl sulfate. Thus the rate of ammonia production from wool protein at 65°C. by hydrochloric acid is less than half the rate found in the presence of sulfuric acid, and this in turn is much less than the rate in the presence of ions of dodecyl sulfuric acid. The anions, much more than H^+ , catalyze hydrolysis by being adsorbed preferentially on the amide groups, and then on the amino groups, of the protein. Normal chains of twelve to fourteen carbon atoms in the case of sulfate half-esters and of twelve or more carbon atoms in the case of sulfonates were shown to have greater affinity for proteins and greater catalytic action on glutamine amide hydrolysis than other isomers or homologues. Steinhardt and Fugitt showed that for wool protein the rapid production of ammonia by this technique corresponded in amount to the glutamic acid present, suggesting that nearly all, if not all, the glutamic acid in this protein existed in the form of its amide, glutamine. In the case of egg albumin the shape of the curve suggested that nearly all the amide present was glutamine. Though Krebs (112), Chibnall and Westall (50), Vickery *et al.* (280), Schlenker (219), and Ferdman (69) had previously determined *free* glutamine and asparagine by measuring the ammonia produced by varying conditions of acid hydrolysis, the technique of Steinhardt and Fugitt measures amides *in* the protein molecule without preliminary hydrolysis. The method of Steinhardt and Fugitt has been extended (9) by the use of a lower temperature and commercially available detergents (Aerosol OT and dodecyl benzenesulfonate) to permit more accurate determination of glutamine and asparagine in proteins such as dog heart muscle proteins, dog plasma proteins, dog hemoglobin, crystalline apoferritin, crystalline catalase, crystalline pepsin, and crystalline trypsin. The results parallel in general those given by Steinhardt and Fugitt (248) for wool protein and egg albumin. Asparagine amide nitrogen, for the time being, was considered to be the difference between total ammonia liberated on acid hydrolysis and the glutamine ammonia determined by the modification of Steinhardt's method. It is probable, however, that this fraction includes at least one and perhaps two sources of ammonia other than asparagine amide nitrogen. Citrulline has been found by Wada (293) in enzymatic digest of casein and by the author (8, 9) in enzymatic digests of plasma protein. Unless this citrulline was synthesized during the enzymatic digestion (as, for example, by enzymatic breakdown of arginine), citrulline must be present in protein. On prolonged hydrolysis of citrulline in boiling strong acid, ammonia is split off. Consequently, citrulline is almost absent from acid hydrolysates of protein. This is one reason why citrulline, like glutamine, has received so little consideration as a component of the protein molecule. It is probable, therefore, that part of the ammonia split off from proteins during hydrolysis by strong acid is derived from citrulline and

does not represent true amide nitrogen. The similarity in structure of the $\text{NH}_2\text{CONH}-$ group of citrulline and the $\text{NH}_2\text{COCH}_2-$ group of glutamine has been discussed by the author (7) in connection with a discussion of the specificity of glutaminase. Despite this resemblance of the terminal amino group of citrulline to amide nitrogen, it does not seem advisable to include this with true amide nitrogen.

During the early stages of the sulfonate-catalyzed hydrolysis of protein at room temperature, the ammonia liberated is almost entirely from combined glutamine. Hydrolysis of peptide bonds, however, is also accelerated, especially in the presence of a high concentration of detergent. The increasingly widespread use of detergents in the study of proteins (264) and in the commercial processing of materials containing protein makes timely a consideration of the rôle of such reactions as were studied by Steinhardt and Fugitt in bringing about the observed alteration in properties of proteins. Sreenivasaya and Pirie (246), working with tobacco mosaic virus protein in the presence of detergents, and Miles and Pirie (155) with the antigen of *B. melitensis*, probably hydrolyzed labile amide as well as peptide groups. After mild hydrolysis with sodium dodecyl sulfate the former workers could no longer precipitate their protein with immune serum. The latter group could no longer precipitate their protein by centrifugation at 16,000 R.P.M. but could precipitate it with immune serum. As pointed out earlier in this paper, a peptide is structurally a substituted amide. Philpot (177) has shown that the hydrolysis reported by Miles and Pirie liberates formic acid and an $-\text{NH}_2$ group which reacts with nitrous acid. As indicated by Lundgren, Elam, and O'Connell (143), alkyl benzenesulfonates form complexes with native and heat-denatured egg albumin on the alkaline side of the isoelectric point. Putnam and Neurath (197) report that horse serum albumin gives anomalous electrophoresis while in complex formation with alkaline dodecyl sulfate. After removal of the detergent the proteins, though electrophoretically homogeneous, had an altered mobility and viscosity. Complex formation of detergents with protein and denaturation occur on both sides of the isoelectric point, but precipitation occurs only on the acid side. The production of fibers from globular protein detergent mixtures (142), the precipitation of soluble proteins by detergent acids (154), the reversible formation of a heme from cytochrome C (106), the dissociation of the chlorophyll-protein complex (244), and the alteration of insulin by dodecyl sulfate (156) are probably all associated to some extent with hydrolysis of amide and peptide bonds and result in a greater or lesser degree of denaturation similar to that demonstrated by Anson (4).

Having now seen that glutamine (and asparagine) are building blocks of proteins, just as we have been accustomed to consider simple amino acids in the past, and having realized that much of the glutamic acid said to be present in proteins is probably present as glutamine, we can understand that there is (a) a plentiful supply of glutamine stored in body proteins, and (b) a need for glutamine or the ability to synthesize it in building body proteins.

Now let us consider the active rôle of glutamine in metabolic processes other than protein synthesis or hydrolysis.

II. THE RÔLE OF GLUTAMINE IN PLANTS

A. THE HIGHER PLANTS

* 1. *Origin of nitrogen of amides*

Since the discovery of asparagine in 1806 by Vauquelin and Roubiquet (269) and the recognition of this substance by Pelouze (175) in 1833 as the amide of aspartic acid, plant physiologists have speculated as to the rôle played by amides in plant metabolism. Their theories and the data from their experiments are worthy of serious consideration by physiologists seeking leads to the study of the rôle of amides in animal metabolism. Studies so far have been based chiefly on the rôle of amides in (a) seedlings and (b) detached leaves. An excellent review on this subject has been published by Chibnall (45). An earlier work by Tauböck and Winterstein (257) reviews not only the physiological rôle of amides in plants but also methods of determination of amides.

As asparagine was isolated seventy-seven years before glutamine, all the earlier work and much of the later work dealt with the more easily recognized asparagine. Since, however, such data as are available point to *similarity of rôles for the two amides in plants, it seems advisable, in consideration of the rôle of glutamine, to review in fair detail the rôle played by asparagine.* This amide was identified and assayed by the method of Hartig and Pfeffer (see 45, page 28). This consisted of dabbing with alcohol a section of tissue on a microscope slide. After the alcohol had evaporated (2 hr.), crystals of the monohydrate of asparagine could be seen under the microscope. Their identity was checked by watching efflorescence at 100°C. and by noting their insolubility in a saturated solution of asparagine.

Piria (178, 179) in 1844 followed the concentration of asparagine in vetch and noted a sharp increase on germination and a decline as the plant matured to and through the fruit-bearing stage. He concluded that the asparagine was derived from a nitrogenous reserve in the plant, but he did not specify protein.

Sullivan (251) in 1858 noted that asparagine accumulated in large amounts in seedlings grown in the dark, and that it disappeared when the plants were exposed to light. Boussingault (26) confirmed these findings in 1868 with the root, stems, and leaves of pea, wheat, maize, and bean. He reasoned that just as animals metabolized protein nitrogen to urea, which they excrete, so plants in the dark metabolize protein nitrogen to asparagine, which remains in the sap until the plant has access to light, which is necessary for utilization of amide nitrogen.

In 1876 Schulze (with Umlauf) published the first of a thirty-five-year series of papers which reflect skillfully conducted experiments, logical reasoning, and conservative speculation. These authors (234) provided the first chemical proof that asparagine nitrogen found in seedlings was derived from protein nitrogen reserves of the seed. Of the protein nitrogen lost from lupin seedlings on germination, 63.5 per cent was present as asparagine nitrogen and free asparagine accounted for 22.3 per cent of the dry weight of the seedlings. Later Schulze and Castoro (233) and Wassilieff (297) found that of the protein nitrogen lost

from *Lupinus albus* during 14 days' growth in light, 86 per cent was present in free asparagine.

Borodin in 1876-78 (21) maintained that while asparagine was formed in all parts of plants it was formed most abundantly and therefore was most easily demonstrated in developing buds and germinating seeds. He concluded that asparagine was produced at a place where protein was decomposed and that its accumulation depended on an inadequate carbohydrate supply, such as would be maintained in buds on cut branches. He regarded asparagine as a transition substance between reserve protein and the cells of the bud or seedling and believed that while asparagine was always being formed it was as rapidly synthesized into protein when an adequate amount of soluble carbohydrate (glucose) was available. Starch in the form of insoluble granules was insufficient, and the amount of monosaccharide required for resynthesis of protein from asparagine was over and above that required by other respiratory processes.

In 1877 von Gorup-Besanez (81) postulated that on germination, enzymatic digestion of reserve proteins of vetch seedlings liberated amino acids and amides. He extracted (80; see also 84) from these seedlings an enzyme which converted albumin to peptone, and he detected leucine, tyrosine, asparagine, and glutamic acid in the germinating seedlings. This list of amino acids was later supplemented by Schulze.

Schulze in 1879 (225) postulated and later (226) proved that glutamine was equivalent to asparagine and played the rôle of asparagine in those plants in which asparagine could not be demonstrated. He recognized (224) also that the proportion of amino acids found free in seedlings was different from that obtained by acid hydrolysis of seed protein, and that the amounts of free amides formed in seedlings were far greater than could exist preformed in seed proteins.

Palladin in 1888 (173) showed that asparagine was not formed when plants were deprived of oxygen. This gave experimental backing to the previous supposition of Pfeffer (176) and Sachsse (210) that asparagine formation from protein involved oxidation. Schulze then argued (227) in 1888 that while part of the asparagine formed in germinating seedlings was probably formed by simple hydrolysis of reserve protein, another part, and probably the largest portion of the asparagine, derived its amide nitrogen from other amino acids liberated by this hydrolysis. That is, he suggested that amino acids liberated by enzymatic hydrolysis of reserve proteins were further degraded in the plant and that their nitrogen was used in asparagine synthesis. In 1892 (228) he suggested that this might be due to oxidation. Prianishnikow (187) in 1899 more eagerly accepted the oxidation hypothesis and pointed out, as had Boussingault (26), that amide synthesis was analogous to production of urea in animals. In 1904 (188) he was convinced that oxidation was involved and gave as evidence Demjanow's *in vitro* oxidation of leucine by permanganate with formation of valeric acid, carbon dioxide, and ammonia, and Butkewitsch's (42) statement that toluene vapor caused cessation of synthetic processes in seedlings while protein decomposition continued with accumulation of ammonia instead of asparagine. By 1906 Schulze (230) conceded that plants oxidatively deaminated amino acids,

and that ammonia, one of the resulting products, then condensed with organic acids to give asparagine or, in certain species, glutamine.^{2,3,4} These amides were then used preferentially by the plant in protein synthesis. The extent of amide accumulation depended on the concentration of glucose available. As had Hlasiwetz and Habermann before him, Schulze believed (230) that both asparagine and glutamine were present in the protein molecule.

Schulze (see 45, page 58) has shown that in seedlings of the orders *Caryophyllaceae*, *Chenopodiaceae*, *Cruciferae*, *Polygonaceae*, *Polypodiaceae*, and *Umbelliferae*, glutamine rather than asparagine predominates as the product of protein metabolism, though the ratio of the two amides varies with age and environment. Oil-bearing seeds (e.g., castor bean) more often yield glutamine. Such seeds have low protein stores, and hence yield little glutamine (229) on germination (2.5 per cent of dry weight). Stieger (249) has indicated the predominating amide in a wide variety of plants and has added iris and carrots to the list of those rich in glutamine. The ratio of glutamine and asparagine in many seedlings has been determined and recorded by Schwab (235). Westall (see 45, page 60) showed that as castor bean seedlings developed, asparagine nitrogen decreased and glutamine nitrogen increased, and that (see 45, page 61) more glutamine is formed than could preëxist in the seed proteins lost. Vickery and Pucher (276) have recently shown that seedlings of summer squash, *Cucurbita pepo*, produce glutamine to the extent of 3 per cent of the original seed weight together with half as much asparagine, and hold out some hope that this may prove a laboratory source of glutamine.

2. Origin of the carbon skeleton of amides

So far we have considered only the nitrogenous precursors of the amides. The carbon skeleton of amides comes usually only in small part from the corresponding amino acids. Amides are often formed from ammonia and a nitrogen-free carbon skeleton derived from glucose. Damodaran and Nair (56) showed that legume seedlings contain *l*-glutamic acid dehydrogenase, through the action of which the glutamic acid formed on hydrolysis of reserve protein is converted to α -ketoglutaric acid. Oxidative decarboxylation gives rise to succinic acid, which may be metabolized through oxalacetic to asparagine.

Chibnall (45, page 95) suggests that arginine, proline, and histidine might also be metabolized to succinic acid, and thence to asparagine. Proline might

* Free arginine, such as occurs in considerable concentration in conifer seedlings, is also a reserve of nitrogen and may be further metabolized if necessary (Mothes (157)). Nearly all the arginine in the seed protein is rapidly liberated to the free form in seedlings (107). There is no good evidence, however, to indicate that it is synthesized by seedlings in preference to amides, as suggested by Suzuki (253). Stieger pointed out (249) that arginine more often occurs with asparagine than with glutamine.

* Some plants with acid sap (pH 1.5-3.0) contain moderately high concentrations of ammonium salts of organic acids as well as varying amounts of amides (126, 207, 208, 209, 235, 281). As pointed out by Vickery *et al.* (281), formation of amides in such plants can scarcely be considered a mechanism for detoxification of ammonia.

* Schulze's ideas on the mechanism of protein metabolism in seedlings are essentially identical with those which are accepted today (see reference 274).

be metabolized either to α -ketoglutaric acid, as by the mechanism shown by Weil-Malherbe and Krebs (302) to occur in kidney, or to succinic acid. Thus either amide could derive from proline. In the case of histidine, the formation of glutamic and succinic acids might proceed according to the path suggested by Edlbacher (59, 60).

Suzuki (252) showed that plants utilized ammonia from their culture medium for asparagine formation more rapidly if glucose was also present in the medium. His findings were confirmed with barley seedlings in 1910 by Prianischnikow and Schulow (190), who added that the ammonia taken up had provided both the amino and the amide groups in the asparagine. Vickery, Pucher, and Clark (278) found the same to be true with respect to glutamine formed in beets grown in plots dressed with ammonium sulfate. These experiments showed for the first time that amide formation was not always secondary to protein hydrolysis. In these cases the amides were derived from nitrogen-free carbon skeletons.

Prianischnikow (189) then showed that plants (lupin) which ordinarily did not form asparagine and which have very low carbohydrate reserves could be made to synthesize amide if the plants were exposed to light so that photosynthesis could supply the necessary carbohydrate. Amide formation also took place (Smirnow (243)), together with protein synthesis, when the lupins were grown in the dark in a medium containing glucose and ammonium salt. Likewise (189), seeds with a high carbohydrate reserve which ordinarily formed asparagine formed none after removal of the cotyledons or endosperms containing this carbohydrate reserve.

Prianischnikow regarded amide formation in plants as a process of detoxification of ammonia. The amides were a non-toxic form in which fixed nitrogen could be stored yet held in readiness for protein synthesis. Ammonia formation he regarded as the first step in protein and amino acid synthesis and the last step in their catabolism.

We have reviewed, so far, data which show that amides are synthesized from a number of amino acids derived from proteins in germinating seedlings. As the protein stores in these seedlings become depleted, the plant relies more on formation of amide (and subsequently on protein) derived from exogenous sources such as ammonia and products derived from glucose. Damodaran and Nair (56) have shown that in some legumes under the influence of an aerobic dehydrogenase *L*-glutamic acid is converted to α -ketoglutaric acid and ammonia. That these products can be converted to glutamine (see 45, page 209) in blades of perennial rye grass and beet roots indicates that the reverse process occurs also in plants. Adler *et al.* (1) have shown that cozymase was the activator of this transamination occurring in higher plants. The fact that protein synthesis (which in growing plants not absorbing amino acids implies amino acid synthesis) takes place readily at the expense of nitrogen stored in amides suggests strongly that the transamination mechanism described (*q.v.*) by Braunstein and Kritzmann (34, 35) for animal tissues, operates also in plants. Euler *et al.* (63) observed that exchange amination took place *in vitro* with glutamic or α -ketoglutaric acid when he used enzyme solutions prepared from higher plants.

Virtanen and Laine (289) demonstrated that aspartic acid (the only α -amino acid found to be produced during nitrogen fixation by bacteria of legumes) transferred its amino nitrogen to pyruvic acid with formation of alanine in the presence of crushed peas. They (285, 286, 288, 289, 290) showed that legume bacteria of peas planted in nitrogen-free sand excreted aspartic acid and its decarboxylation product, β -alanine, from root nodules. Virtanen and Laine (287) postulated that nitrogen fixation took place through formation in series of hydroxylamine, oxalacetic acid (later (289) shown to be present), oxalacetic acid oxime, and *l*-aspartic acid. *Azotobacter chroococcum* and *Beijerinckia* were shown to yield oxime nitrogen (62, 287) and aspartic acid (287). These authors concluded that other amino acids may then be synthesized at the expense of aspartic acid or asparagine.

Let us consider again the possible and likely precursors of the nitrogen-free skeletons of the amides. One may postulate from analogy (as has Chibnall (45, page 190)) that, as in muscle metabolism (3, 83, 306), enzyme systems are present which convert succinic acid through fumaric and *l*-malic acids to oxalacetic and aspartic acids and that each of the four nitrogen-free acids (as is the case in muscle) (3, 247, 255) catalyzed oxidation.

Just as such reactions could provide the precursors of asparagine, so citric acid or *cis*-aconitic (38, 117) through the cycle of Krebs (114) and Johnson (120) could lead to the formation of α -ketoglutarate (146, 147), glutamic acid, and glutamine. Indeed, some of the suggested precursors of asparagine (e.g., oxalacetic acid) are believed to be converted by muscle to citric or *cis*-aconitic acid through oxidation of carbohydrate according to the mechanism of Knoop and Martius (109) and might therefore act also as precursors of glutamic acid and glutamine.

Buchanan *et al.* (40) have recently found that heavy carbon in acetoacetic acid after treatment with homogenized kidney cortex can be recovered in α -ketoglutaric and fumaric acids. This suggests that in animals, acetoacetic acid and substances which are metabolized to it are to some extent at least precursors of α -ketoglutaric acid and therefore also precursors of glutamic acid and glutamine.

Experimental data to indicate whether or not such a mechanism operates in plants are still meagre and fragmentary. Virtanen and Tarnanen (291), however, have claimed that there is aspartase activity in germinating peas and in leaves of red clover.

Chibnall suggests that the nitrogen-free precursors of the dicarboxylic amino acids and their amides might be derived from carbohydrate, fat, or protein (45, page 193). Thus proteins, after hydrolysis to amino acids and deamination, give rise to α -keto acids which may condense with oxalacetic acid to give citric acid or be oxidized to succinic acid. Fats would give rise to succinic acid (270), and carbohydrates could provide the necessary precursors through the action of Krebs's cycle. Conversely, Chibnall postulates that asparagine and glutamine are reservoirs of oxalacetic acid and α -ketoglutaric acid for use by plants during seasons of extreme starvation. Suggestive, but as Chibnall (45, page 197)

points out, not conclusive evidence that these organic acids were precursors of amides was given by Mothes (158) in 1933, using vacuum infiltration of leaves with ammonium salts of organic acids by the method of Björkstén (18). However, the work of Schwab (235) and of Chibnall (45, page 205) failed to confirm these observations. Chibnall believes, nevertheless, that when solutions of ammonium α -ketoglutarate are infused, both ions are used for glutamine synthesis by rye grass. However, definite proof of the exact nature of the nitrogen-free precursors of the amides will probably wait for application of a new technique, such as the use of metabolites containing tagged atoms. It seems reasonable to assume, however, that when ammonium ions and oxalacetic or α -ketoglutaric acid are present they would be used for synthesis of asparagine and glutamine equally well, whether the nitrogen-free precursor be derived from carbohydrate, fat, or protein, or whether it be poured preformed into the system from a test tube.

Whether the nitrogen-free precursors of amides derive from carbohydrate, fat, or protein, or whether they are preformed in the plant, would seem to depend on the history and environment of the organism. Under given conditions one food type may supply the precursor and under others another may provide the carbon skeleton of the amides. It would seem that there was a dynamic equilibrium of metabolites and products, and whether amide formation took place at the expense of exogenous nitrogen or ammonia from breakdown of proteins on the one hand, and on the other hand from carbon skeletons from proteins, carbohydrates, or fat, would depend on the age of the plant, its rate of growth, the relative and absolute concentrations of different types of food stores, and physical conditions such as light, temperature, humidity, etc. If the experimenter were to raise the concentration of an exogenous amide precursor and obtain increased yields of amide, he would indicate thereby neither whether this mechanism were one by which the organism ordinarily synthesized the major portion of its amide, nor whether the nitrogen-free precursor ordinarily was derived from carbohydrate, fat, or protein.

3. *Amide metabolism in detached leaves*

In barley leaves which have been detached from part of the plant, part of the leaf protein disappears. Simultaneously the concentration of glutamine increases. Glutamine nitrogen in detached barley leaves comes probably in part from direct hydrolysis of protein. However, the increase in free glutamine nitrogen is so much greater than the amount of amide nitrogen which is present in the leaf protein which is hydrolyzed that at least 75 per cent of the free glutamine formed must derive from other amino acids (308). As the concentration of carbohydrate decreases, first a stable amide and then free ammonia forms. In detached tobacco leaves, Pucher, Vickery, *et al.* (193, 194, 195) noted that asparagine formation was rapid in the dark but retarded in the light. Glutamine formation, however, was rapid in the light and very slow in the dark. Vickery (273) and Chibnall (45, page 224) have shown that the nitrogen for amide formation was made available through oxidation of amino acid to give ammonia.

Vickery (271) and Vickery *et al.* (282) showed that in detached tobacco leaves glutamine was formed in the light only when products of photosynthesis (carbohydrate) were available, suggesting that carbohydrate was the precursor of the carbon skeleton of glutamine. Asparagine, however, was formed whether or not light and carbohydrate were available, and since in the light the concentration of free malic, citric, and oxalic acids in the leaves remained unchanged, Vickery *et al.* felt that these acids were not the nitrogen-free precursors of asparagine. In the dark (when carbohydrate was used up), however, the concentration of malic acid decreased, and they felt that malic acid might have been utilized in asparagine formation, despite the fact that the malic acid loss was associated with an increase in citric acid in the dark only (271).

Rhubarb leaves (272, 275, 283, 284), on the other hand, after 114 hr. in the dark exhibited marked glutamine synthesis without formation of asparagine. Addition of glucose did not stimulate amide synthesis, and the same yield of glutamine (7 per cent of organic solids in some specimens (281)) was formed on exposure to light (277). Fifty per cent of the soluble nitrogen was present as ammonia. Vickery concludes that in excised leaves protein is metabolized through amino acids and ammonia to the amides.

When *Lupinus augustifolius* seedlings are grown in the dark, asparagine concentration reaches a maximum (11 per cent of original seed weight) after 12 days, and then drops rapidly with production of ammonia. Since no invasion by microorganisms could be demonstrated, Vickery and Pucher (276) concluded that this change reflected an exhaustion of non-nitrogenous components essential for synthesis of asparagine.

Mothes (159) notes that although starved plants do not either make or contain glutamine they maintain the mechanism for synthesizing it. As long as carbohydrate is present, a given plant maintains a constant glutamine/asparagine ratio.

4. Presence of amide hydrolase in plants

The enzymes involved in transfer of amide nitrogen to and from the carboxyl group of dicarboxylic acids have been demonstrated in several plant tissues. Chibnall and Grover (49) prepared asparaginase from germinating barley. Mothes (158) showed that leaves synthesize asparagine from ammonium aspartate. Vickery *et al.* (277, 279) and Chibnall (45, page 205) have shown that tomatoes, beets, and perennial rye grass (*Lolium perenne*) synthesize glutamine (the latter two at least from ammonium glutamate), and concluded that roots of these plants contain a rapidly synthesizing glutaminase. Vickery *et al.* (279) note that in beets grown in soil dressed with ammonium sulfate the increase in soluble nitrogen corresponds to both nitrogens of glutamine. Hence ammonia appeared to serve as a source of nitrogen for both the α -amino and the acid amide nitrogen of the synthesized glutamine. These workers nevertheless assumed that the synthesis of glutamine took place in two steps: (1) formation of glutamic acid from a non-nitrogenous compound; (2) dehydration of ammonium glutamate to glutamine by a mechanism such as that shown by Krebs to take place in some

animal tissues. Chibnall and Schwab interpreted this as indicating that beets contained glutaminase. Indeed, Schwab (235) suggested that plants be characterized as glutaminase or asparaginase plants, rather than as glutamine- or asparagine-forming plants.

Wood and his colleagues (303, 304, 305) have shown that glutamine rapidly disappears from fasting leaves of Kikuyer grass and that asparagine accumulates. In leaves of Algerian oats (*Avena sterilis* L.) glutamine accumulates. Wood assumes that the synthesizing enzyme for the amides is present in the leaves of these plants.

The author has found (7, 9) that tomato, zucchini, and perennial rye grass contain small but demonstrable concentrations of an enzyme which *hydrolyzes* glutamine to ammonia *in vitro*. The concentration of this enzyme is increased greatly if the plants are grown in soil dressed with ammonium glutamate. The enzyme is localized to the roots. Geddes and Hunter (74) claim that yeast extract can hydrolyze either amide, although Nielsen (167) and Schwab (235) report that it acts only on asparagine. Grassmann and Mayr (82) and Luck (141) claim that yeast extract hydrolyzes glutamine. The author, using a baker's yeast or brewer's yeast, found no action on glutamine and very little on asparagine until the yeast had been cultured in a medium containing asparagine (9).

In summary, we may conclude that reserve proteins of plants (as, for example, in germinating seedlings) are capable of being hydrolyzed enzymatically to amides and amino acids. The amino acids may be used in part for resynthesis of protein, but in part are deaminated, and from their products new amino acids, asparagine and glutamine, are formed, all these products being capable of use for protein synthesis. Amides tend to serve as a neutral store of fixed nitrogen, especially (a) when conditions are such as to favor inadequate concentrations of nitrogen-free precursors of amino acids, as, for example, when the light intensity is low enough to prevent appreciable photosynthesis of carbohydrate, and (b) when plants starve and proteins must be metabolized to supply energy to the plant. On the other hand, proteins of growing parts have different amino acid compositions from the proteins of reserve organs; hence in the absence of adequate external sources of nitrogen, the requisite amounts and kinds of amino acids tend to be synthesized by the plant from reserves, presumably with involvement of amide nitrogen. This synthesis of new amino acid and protein from amide is favored when adequate carbohydrate is made available from "stores" or by photosynthesis.

The direction of these several reversible reactions—hydrolysis, synthesis, transamination, etc. (catalyzed by enzymes)—and the position of the dynamic equilibrium established are governed by mass-action laws and relative concentrations of the reactants. These concentrations in turn vary with physical and chemical characters of the environment. As Chibnall has pointed out (45, page 112), the observation by Braunstein and Kritzmann that aspartic and glutamic acids can donate amino nitrogen to α -ketonic acids serves to emphasize the ability of the corresponding amides to serve as reserve agents for protein synthesis.

A relation between carbohydrate metabolism and protein synthesis is seen when it is considered that carbohydrate provides the necessary α -keto acids for formation of amino acid units of protein structure as well as the energy necessary for amide and protein synthesis.

B. THE LOWER PLANTS

1. *Glutamine in the physiology of bacteria*

Since 1881, when Loeffler introduced the use of meat extract in culture media, bacteriologists have been aware that the extract contained an elusive factor which was necessary for the growth of certain pathogenic organisms. McIlwain reported that the factor in this extract was glutamine and isolated glutamine from horse meat. In 1939 McIlwain *et al.* (153) reported that most, though not all, of the pathogenic strains of *Streptococcus hemolyticus* required glutamine for their growth. As little as $M/500$ glutamine added to the inadequate basal culture medium permitted full growth in 16 hr. In the same year Fildes and Gladstone (70, 71) reported that Group A hemolytic streptococcus as a rule required glutamine, while Group B streptococcus usually grew as rapidly without its addition. Most of the strains of Groups C, E, F, and G required glutamine. McIlwain (152) showed that no compound, even though closely related chemically, could replace glutamine. He reasoned that since insulin is not a substitute for glutamine in the growth of *Streptococcus*, the organisms do not break down glutamine peptides to glutamine, and that it is unlikely that the organisms have (reversible) enzymes present capable of synthesizing these peptides (unless the enzymes present are specific for the peptides involved). McIlwain felt that the function of glutamine is for ammonia transference rather than for protein synthesis. This is in contrast to the belief of Pollack and Lindner (183) regarding the function of glutamine in the case of lactic acid bacteria (*q.v.*). McIlwain showed that growth of streptococci took place, though less actively, if glutamate (in concentration 100 times greater) were added instead of glutamine, and assumed that glutamine was synthesized from glutamate. Landy (127) confirmed McIlwain's findings but added that in peptone there is present another factor necessary for optimum growth of *Streptococcus hemolyticus*. Fildes and Gladstone in 1939 (71) reported that of six strains of *B. anthracis* tested, five grew more rapidly in the presence of glutamine. All of four strains of *Streptococcus viridans* required glutamine. Glutamine and glutamic acid produced equal but marked stimulation of growth of *Proteus* but asparagine had no effect. Glutamine stimulated growth of *E. coli* when the inoculum was a 14-day-old culture, but not when it was only 24 hr. old. Glutamine had little if any stimulating effect on the growth of *H. influenzae* or *C. diphtheriae* and none on *N. gonorrhoeae*.

Lankford and Snell (129), however, report that glutamine is necessary for certain strains of *Neisseria*. Fildes and Gladstone claim that *Staphylococcus* showed some acceleration of growth on addition of glutamine. One strain of *Pneumococcus* tested gave much more rapid growth when glutamine was present. A high concentration of glutamate, however, caused good growth in the absence

of glutamine. Bernheimer and Pappenheimer (16, 17) in 1942 showed that a concentration of 10 mg. per cent glutamine gave 75 per cent of maximum growth of *Pneumococcus*, and used 20 mg. per cent glutamine in their medium for *Streptococcus hemolyticus*. Gibert (76) has studied the effect of varying concentrations of glutamine and asparagine on the growth of *Pneumococcus*, and has noted that 10 mg. of glutamine per 100 cc. inhibits some strains and favors the growth of other strains of *Pneumococci*. Strong, Feeney, and Earle (250) and Feeney and Strong (66) reported that glutamine in a concentration of 0.01 mg. per 100 cc. was effective as a growth factor for *Lactobacillus casei* and assumed that the organisms are capable of synthesizing glutamine from glutamic acid. Asparagine was also an effective stimulant but chiefly when glutamic acid was present to accept the amide nitrogen.

Pollack and Lindner (182, 183) reported the stimulating action of glutamine and many plant and animal extracts on the growth of lactic acid bacteria (including *Streptococcus lactis* and *Lactobacillus arabinosus*). Addition of other amino acids was without effect. Known vitamins were present. Glutamine gave a response ten times as strong as did peptone. Lewis and Olcott (137) found that glutamic acid or glutamine was necessary for the growth of *L. arabinosus* 17-5. Glutamine was 140 per cent as effective as glutamic acid. Lyman *et al.* (144) observed that production of lactic acid by this microorganism (used as a measure of growth of the bacteria) did not increase with increasing amounts of glutamic acid until a concentration of 0.8 mg. of glutamic acid per 100 cc. of medium had been reached. When 0.2 mg. of glutamine was present in 100 cc. of medium the increase in the lactic acid formed in 72 hr. was almost proportional to the concentration (from 0.0 to 1.2 mg. per 100 cc. of medium) of glutamic acid added. They conclude that glutamic acid is first converted to glutamine by the organism. This contention is supported by their observation that in the presence of glutamic acid, but only in the absence of glutamine, addition of ammonium salts increases the production of lactic acid. This further suggests that the organism contains an enzyme system capable of synthesizing glutamine from glutamic acid and that the chief use of glutamic acid, in the economy of this organism's metabolism, is for the synthesis of glutamine; probably the glutamine is used for synthesis of cellular proteins (*cf.* 84a). Pollack and Lindner (182, 183) believe that there is also an unknown stimulating factor other than glutamine in natural products. This unknown has an isoelectric point between 3.5 and 4.5, is not inactivated by proteolytic enzymes, and is less easily hydrolyzed than glutamine. Unlike glutamine, it can withstand heating for 2 hr. at 100°C. at pH 2-11. They believe that the material is not a peptide of glutamine, since enzymatic digests of the peptone factor grow even more rapidly when glutamine is added. Later Pollack and Lindner stated that nine strains of lactic acid bacillus require either glutamic acid or its amide. Four require glutamine (or eleven times as much glutamic acid). Ammonium chloride was not sufficient. They believed that the glutamate or glutamine is required simply as building blocks for the formation of cell protein.

Niven (168) notes that for maximum growth of *Streptococcus lactis* and *Strep-*

Staphylococcus cremoris either glutamine or asparagine (and also valine, leucine, isoleucine, methionine, and arginine) is required.

Hill and Mann (96) reported that the inhibitory effect of sulfanilamide on *E. coli* growth *in vitro* was counteracted by glutamine or glutamate concentrations ranging from 15 mg. per cent to 150 mg. per cent. Harris and Kohn (93) had previously reported that methionine (especially in the presence of xanthine and hypoxanthine) offered similar protection of *E. coli* from sulfanilamide.

Bovarnick (27, 28, 29) has demonstrated that when glutamate and asparagine (0.007 *M*) react at 100°C. and pH 7.0 for 24 hr. some nicotinamide is formed. The reaction is catalyzed by 7×10^{-5} *M* ferrous sulfate. The same reaction takes place though to a smaller extent when glutamine is heated. One wonders whether or not the synthesis of nicotinamide from amide and glutamic acid accomplished *in vitro* by such drastic means can be catalyzed enzymatically *in vivo*.

The phenomenon reminds one of the rather surprising claim of Fildes *et al.* (see 152) that glutamine in some bacteriologic culture media containing agar was stable to autoclaving at 120°C. and pH 7.2 for 20 min., and that only a little loss of activity occurred when meat concentrate was heated at pH 5, 6, or 7 at 100°C. for 1 hr. It is likely that if glutamine is added to the culture medium before autoclaving a small amount of nicotinamide is formed during the autoclaving. It is unlikely, however, that such a reaction accounts for the activity of autoclaved glutamine, when nicotinamide is added to the basal medium. On the other hand, it is possible that glutamine in the presence of the components of the culture medium or at the pH of the medium is adequately (though only partially) protected from decomposition by heat. Niven (168) notes that asparagine and glutamine are more effective if they are sterilized by filtration rather than by autoclaving, and Lankford *et al.* (128) note that the factor necessary for growth of gonococci is destroyed on autoclaving. This is to be anticipated in view of the heat lability of glutamine especially.

Tatum *et al.* (256) noted that glutamine or asparagine was necessary for the fermentation of starch by butyric acid bacteria.

2. Yeast

Nielsen (167) has shown that glutamine is not an essential component of media for the strains of yeast he tested. Smythe (245) has shown that glutamine increases the rate of fermentation by yeast.

The above observations indicate that glutamine is an essential metabolite in the more specialized of even the lowest forms of plant life. Some forms cannot grow unless glutamine is already present. Others appear to be able to synthesize the necessary glutamine if the proper precursors are present. Whether this glutamine is used merely in building proteins of the bacterial cell, or whether it is used also, or only, as a carrier of labile —NH_2 , is a matter which must await further study.

III. THE RÔLE OF GLUTAMINE IN ANIMAL METABOLISM

Krebs (112) reported that there were present in the liver, kidney, brain, and retina enzymes which were capable, on the one hand, of synthesizing glutamine

from glutamic acid, and on the other, of hydrolyzing glutamine to glutamic acid. From this it seemed reasonable to suppose that glutamine played an essential rôle in animal metabolism in each of these three sites, and that at least part of these essential reactions was localized to the organs mentioned and that the glutamine might play different rôles in the several organs.

It would not be surprising then to find considerable concentrations of glutamine either in these organs or in the circulating fluids, including blood, or in both. The concentrations expected would vary depending upon the summation of the relative rates of hydrolysis and synthesis under the several conditions to which the tissue had been subjected prior to analysis. The concentration would tend to be high in those organs the enzyme systems of which favored synthesis or storage and low in the organs which favored glutamine hydrolysis. Let us consider the rôles in blood and in each organ named.

A. PRESENCE OF GLUTAMINE IN BLOOD

Glutamine, or a substance very closely resembling it in properties, has been reported by Ferdman, Frenkel, and Silakova (69) to be present in dog and pigeon blood in concentrations of 14 and 20 mg. per 100 cc., respectively. These authors measured glutamine by determining the amount of ammonia set free on heating for 5 min. at 100°C. with 5 per cent sulfuric acid. After these findings had been published and just before they became available in this country, Hamilton and the author, working in the same laboratory but using different methods, concluded that 5-12 mg. of glutamine was present in 100 cc. of fasting dog or human plasma. Hamilton (85, 268) used the ninhydrin carbon dioxide method. The author (6, 7, 268) used a glutaminase method. Hamilton concludes that red cells (86, 87) have approximately the same concentration of glutamine as the surrounding plasma. Harris *et al.* (89, 91) have followed the rate of ammonia liberation on acid hydrolysis at various temperatures and concluded that the glutamine levels reported by Hamilton hold for both human plasma and spinal fluid, and that no appreciable concentration of asparagine is present. The fact that Cohen (51) finds human plasma has a total of 3 mg. per 100 cc. glutamic acid plus glutamine by a method which does not distinguish between the two compounds, and that 5-10 mg. per 100 cc. of glutamine is found by several laboratories and methods indicates that nearly all, if not all, the glutamic acid in blood is present as the amide.

It is our belief (6) that spontaneous breakdown of glutamine in plasma is responsible for part of the " γ -ammonia" which Conway and Cooke (53) report is liberated from plasma. Whether or not glutamine is the precursor of the ammonia formed in the kidney and liberated into the renal venous blood is still undetermined. It would not be surprising, however, if this ammonia were formed by the same mechanism that provides most of the urinary ammonia in dogs (268).

The above-mentioned workers (6, 7, 69, 85, 89) and Gerard (75) favor the hypothesis suggested by Leuthardt (109) that glutamine serves as an ammonia carrier in cell metabolism. Örström, Örström, and Krebs (169) suggested that it acted specifically as an ammonia carrier in formation of hypoxanthine in pigeon liver.

B. PRESENCE OF GLUTAMINE IN CEREBROSPINAL FLUID

Values given by Ferdman *et al.* (69) indicate a concentration in the human spinal fluid of 1.0 mg. of glutamine amide nitrogen per 100 cc. This figure corresponds to the value of 5–10 mg. of glutamine per 100 cc. of spinal fluid given by Harris (89).

C. PRESENCE OF GLUTAMINE IN JOINT FLUID

Glutamine concentration in human joint fluid (6) appears to parallel that in blood plasma.

D. PRESENCE OF GLUTAMINE IN URINE

So far there are few data to indicate the concentration of glutamine in urine. Cohen (51), using a technique which does not distinguish between glutamine and glutamic acid, found that the sum of the two amounted to 4.3 mg. per 100 cc. in human urine. McIlwain (153) concludes that because urine supports the growth of *S. hemolyticus* it contains glutamine, but he does not indicate the concentration. Ferdman *et al.* (69) report that 1.4–2.4 g. of glutamine per 24 hr. is excreted in human urine. As pointed out by Hamilton (86, 87), this figure is probably too high, as Ferdman *et al.* estimated glutamine amide nitrogen as the ammonia liberated by hydrolysis for 5 min. at 100°C. with 5 per cent sulfuric acid. The relatively high concentration of urea in urine would contribute appreciable ammonia under these conditions. Enzymatic determination (6, 7, 9) of glutamine in one normal human urine (sp. gr. 1.026) indicated a concentration of 4.7 mg. of free glutamine per 100 cc., a figure which is very close to that given by Cohen for glutamic acid (plus glutamine). Such data as are available (6, 9, 22, 51) indicate that arginine, glycocyamine, glutamic acid, and glutamine spill through into the urine in roughly the same relative proportions as exist in plasma. Some glutamine may be present in human urine combined with phenylacetic acid.

E. PRESENCE OF GLUTAMINE IN EGG

The "white" of unincubated hen's egg contains only a very low concentration of glutamine. The yolk, however, contains relatively large amounts of free glutamine (6). On incubation of eggs the total amount of free glutamine decreases and the concentration of glutamine outside the yolk increases (9). Ultraviolet-absorption curves of dialysates (9) of portions of incubated and unincubated eggs indicated the absence of appreciable concentrations of interfering substances such as adenosine or adenylic acids which, if present, would increase the apparent concentration of glutamine.

The presence of a high concentration of free glutamine in egg and the maintenance of a moderately high concentration during incubation of fertile eggs is probably one reason why the egg is such an excellent medium for the growth of certain microorganisms. Presumably the free glutamine concentration in in-

cubating eggs is maintained as part of a dynamic equilibrium between the bound glutamine of protein reserves and of embryo protein. Whether or not there occurs in the egg a synthesis of glutamine from other amino acids similar to that which takes place in seedlings has not yet been determined.

F. RÔLE OF THE GLUTAMINE-GLUTAMIC ACID-GLUTAMINASE SYSTEM IN THE CENTRAL NERVOUS SYSTEM

A better understanding of the significance of glutamine as a metabolic product and constituent of nervous tissue will be obtained if preliminary consideration is given to the rôle of a metabolite which probably stimulates glutamine formation, *viz.*, ammonia.

Work on the physiology of ammonia in nervous tissue up to 1935 has been reviewed by Schneller (221) and will not be discussed further in the present review. As shown by Pugh and Quastel (196), ammonia is formed by isolated brain from butyl-, amyl-, isoamyl- and heptyl-amines, and amines decrease the oxygen consumption of brain.

In 1935 Kahn and Chekoun (104) showed that by partial asphyxiation of fish, the ammonia production by brain was increased. Increased ammonia production occurred also *in vitro* on stimulation of brain. Formation of unusually large amounts of ammonia in nerve tissue of molluscs was noted by Kahn *et al.* (77, 103).

Sapirstein *et al.* (215, 216) reported that 0.5 g. of ammonium salts given by mouth to humans increased "after-contractions" which they regarded as a measure of cerebral cortex excitability. Bruhl (39) stated that the ammonia content of brain increased as the adenylic acid content decreased in convulsive states.

Krebs (112) in 1935 showed that brain contained glutaminase and that this enzyme *in vitro* synthesized glutamine from ammonia and glutamic acid when an energy source such as glucose was present. The following year he showed that addition of *L*-glutamic acid to brain doubles the QO_2 and the brain converts free ammonia nitrogen to amide nitrogen. Grey cortex and retina can bind ammonia at the rate of 0.8 per cent of the tissue dry weight per hour if glutamic acid is present.

Weil-Malherbe (299, 300) later in the same year reported that of twelve amino acids tested, glutamic acid was the only one which caused increased uptake of oxygen by brain tissue *in vitro*. Both *L*- and *D*-forms were oxidized. Later (301) he stated that in brain (and spleen) 0.033 *M* ammonium ion inhibits anaerobic glycolysis but increases aerobic glycolysis. Glutamate, hydroxyglutamate, and glutamine have the same effect on brain (only), especially in the presence of 0.01 *M* succinate or lactate. Harris, Blalock, and Horwitz in 1938 (92) suggested that the drop in blood amino acid level noted by them during insulin hyperglycemic shock therapy of mental patients had an effect on the mechanism referred to by Krebs. No data were given in support of this view. Harris (90) in 1943 showed that glutamine participated in this fall of blood amino acid level during insulin shock.

Folch-Pi (73) in 1942 postulated that glutamic acid under the influence of

glutaminase detoxified the ammonia formed in nerve tissue and that in the brain glutamine was a by-product of this detoxification. Sapirstein (214) in 1943 evoked convulsions in twelve rabbits by injecting 15-25 cc. of 2.5 per cent ammonium chloride at the rate of 1 cc. per kilogram per 5 min. Complete protection from these convulsions was given by a preliminary injection of 50 cc. of 5 per cent *l*-glutamic acid in 3 per cent sodium bicarbonate, even when 50 cc. of 2.5 per cent ammonium chloride was given. Aspartic acid failed to offer protection.

Price, Waelsch, and Putnam (191) report that on feeding 12 g. of *dl*-glutamic acid hydrochloride to persons who are subject to frequent attacks of psychomotor disturbance or *petit mal*, the number of attacks is decreased from 25-50 per day to 5-25 per day, as long as the medication is continued. They attribute the effect chiefly to acidification of the fluid about the brain cells, but used glutamic acid because of the unique rôle which the *l*-form is believed to play in brain metabolism. Later Waelsch and Price (295) concluded that *l*(+)-glutamic acid was the chief factor favoring improvement. Decision as to whether the phenomenon produced by administration of glutamic acid was the result of an increased rate of detoxification of ammonia and synthesis of glutamine (a reaction which is retarded *in vitro* by the addition of either *d*- or *l*-glutamic acid) or whether it is a result of activation of the synthesis of acetylcholine by *l*(+)-glutamic acid (294) must await further work. Atebrin strongly inhibits glutaminase (7) and choline esterase (294). Whether its action on the esterase is due to inhibition of glutamine hydrolase and a decreased concentration of glutamic acid, is undetermined.

Since glutamine is less than half as active as glutamic acid as an accelerator of acetylcholine synthesis by rat brain extract (160, 294), it is conceivable that the glutamine-glutaminase system in brain (by controlling the relative concentrations of glutamic acid and glutamine) is involved in a control of choline acetylase activity and therefore also in the control of the potential of nerve action.

Hueber (98) has reported that administration of 10 cc. of 10 per cent magnesium glutamate three times a day to hyperthyroid patients having a basal metabolic rate 25-60 per cent above average reduced the basal metabolic rate until it was only 7-13 per cent above average. No clinical improvement accompanied this fall. Decision as to whether or not this observation is dependent on the action of glutamate on the nervous tissue must await further investigation.

G. RÔLE OF GLUTAMINE AND GLUTAMIC ACID IN MUSCLE METABOLISM

Riabinovskaja (199) reports that in a medium of 0.01 *M* sodium glutamate the indirect excitability (i.e., through the nerve) of frog sartorius-nerve preparations is eliminated and the direct excitability is decreased. The presence of high concentrations of both glutamine (69, 87, 153) and adenosine derivatives in muscle has not yet been shown to be more than fortuitous but suggests that the two components may function in the same system. The subject of transamination in muscle (52) will not be discussed here, since similar processes in the liver are considered in the next paragraph.

H. RÔLE OF GLUTAMINE IN LIVER

1. Deamination-transamination

The liver has long been regarded as the chief center of deamination of amino acids. The subject of transamination has been discussed thoroughly by Herbst (95) and is reviewed here only insofar as it relates to the dicarboxylic acids and their amides. While the reactions of transamination require glutamic or ketoglutamic or the corresponding aspartic (31) compounds and have not been shown to require participation of the amides, the significance of glutamine in relation to transamination and deamination is that it can act either as a storage or transport form of α -amino nitrogen, glutamic acid, or α -ketoglutaric acid. That is, glutamic acid may accept the $-\text{NH}_2$ made available by the deamination of other molecules, thus forming glutamine (112). This glutamine is in part then transported from the liver to other parts of the body *via* blood to perform other functions elsewhere and may be, in part, utilized directly in the liver to form urea (116, 130, 131, 135, 136).

In 1939 Karyagina (105) showed that *in vitro* skeletal and heart muscle, liver, kidney, and brain, the amino groups of glutamic and aspartic acids in the presence of pyruvic acid were transferred, giving rise to alanine and α -ketoglutaric and oxalacetic acids. Kritzmann (123) showed that this and the reverse action occurred also *in vivo* in rabbits, pigeons, and white mice. Bychkov (43) showed that where the carboxyl group of a monocarboxylic amino acid is supplemented by a second acid group ($-\text{SO}_3\text{H}$ as in cysteic acid or $-\text{OPO}_3\text{H}_2$ as in phosphoserine), the resulting acid is capable of donating $-\text{NH}_2$ to a keto acid just as do either of the two dicarboxylic amino acids, but cannot catalyze the transfer of $-\text{NH}_2$ between two monocarboxylic acids such as lysine and pyruvic acid. Braunstein and Kritzmann (36, 37) showed that there is no transfer of $-\text{NH}_2$ from monocarboxylic acids to keto acids unless $M/16,000$ of either aspartic or glutamic acid or their precursors (keto acids or citric, succinic, fumaric, or maleic acid) were present. Braunstein and Bychkov (33) maintained that deamination of *l*-monocarboxylic amino acids involved first a transfer of the α -amino group to ketoglutaric or oxalacetic acid by the corresponding enzyme, glutamic or aspartic "aminopherase"⁵ (31), and that aspartic or glutamic dehydrogenase then deaminated the dicarboxylic amino acids (see also 32). Both aminopherases have been purified and require a coenzyme (121, 122, 124). Kritzmann (36) reported that of the amino acids tested all except glycine are deaminated by this mechanism. Cohen (52), on the other hand, reports that in pigeon breast muscle only aspartic acid and alanine readily donate their $-\text{NH}_2$ group to α -ketoglutaric acid to form glutamic acid. Conversely, the corresponding deamination products, oxalacetic and pyruvic acids, most readily accepted $-\text{NH}_2$ from glutamic acid. α -Aminobutyric acid and valine were slightly active as $-\text{NH}_2$ donors to ketoglutaric acid, but seventeen other amino acids were inactive.

Braunstein and Bychkov (33) accomplished *in vitro* aerobic deamination of

⁵ The term "transaminase" has been used by some English and American writers to include both the aminopherase and dehydrogenase referred to by Braunstein.

l-alanine with the cell-free system including glutamic aminopherase and dehydrogenase, cozymase and ketoglutaric acid and an autoxidizable hydrogen carrier such as methylene blue. The aminopherase was inhibited by 0.001 *M* potassium cyanide, quinone, or glutathione.

Cedrangolo and Carandante (44) are reported (44b) to have stated that *l*-amino acid oxidase may be identical with glutamine aminopherase and that the indirect oxidation of *l*-amino acids which involves amination of ketoglutaric acid occurs chiefly in the kidney, while the main oxidation is accomplished directly by another enzyme. According to Neber in 1936 (164) proline is broken down by a different mechanism and in the liver is oxidized to glutamic acid. He showed that in the presence of hydrogen peroxide proline is oxidized to glutamic acid.

I. RÔLE OF GLUTAMINE IN UREA FORMATION

From data available at present it would appear that (1) some urea is synthesized by operation of the ornithine-arginine pathway suggested by Krebs but that one step—the conversion of citrulline to arginine—can take place in the kidney, and (2) urea can be synthesized in the liver by a mechanism which utilizes amide nitrogen and which is independent of the ornithine mechanism.

In the present review Krebs's mechanism will be considered only to such an extent as will facilitate interpretation of experiments dealing with the rôle of glutamine in urea synthesis.

1. *Krebs's mechanism*

Since Krebs (119) postulated the ornithine-citrulline system for urea formation, there has been considerable doubt as to whether the mechanism, as he outlined it, represents the main or whole pathway of *in vivo* synthesis of urea. Such evidence as there is to support Krebs's theory can be summarized as follows: (1) The formation of urea from ammonia by liver slices or perfused liver (263) is catalyzed by ornithine, and the process has been shown by isotopic studies (64, 201) to be accompanied by an uptake of carbon dioxide. (2) When isotopic nitrogen was fed to animals as ammonia and amino acid, more was recovered in the amidine group of arginine in liver proteins than in any other form (though much isotopic nitrogen turned up in the "amide nitrogen" liberated as ammonia during acid hydrolysis of the tissue proteins) (222). It is conceivable that free liver arginine and arginine in liver proteins continuously exchange places (222). (3) Citrulline, which according to the theory is an intermediary product, has been isolated from liver (78) and has been found in blood (8). (4) Arginase, which hydrolyzes arginine to urea and ornithine, is found only in livers of those animals whose main end product of nitrogen catabolism is urea (100, 101).

Gornall and Hunter (78) have observed that although completion of Krebs's chain of reactions can take place in liver slices, the rate of formation of citrulline is considerably more rapid than the rate of conversion of citrulline to arginine. Recent work of Borsook and Dubnoff (22), confirmed by Krebs (116), indicates that the enzyme systems necessary for the conversion of citrulline to arginine are present in the kidney (79). Citrulline, which Krebs suggested (119) and

Gornall and Hunter (78) demonstrated, is formed in the liver, may be converted to arginine partly in the liver and partly in the kidney. It has not yet been determined what percentage of the conversion of citrulline to arginine normally occurs in liver and what fraction occurs in kidney. In kidney tissue suspensions either aspartic or glutamic acid or their amides could serve as the donor of —NH_2 to citrulline to form arginine (22). No other amino acid (except proline, hydroxyproline, lysine, and ornithine, which are believed (22) to be converted to dicarboxylic acids) can replace the dicarboxylic acids in the formation of arginine from citrulline in kidney. In the case of the animal tissues tested, the dicarboxylic acids did not appear to serve the same function in the liver.

Whether or not glutamine has a significant rôle to play in the operation of Krebs's chain is still a matter of debate. It may donate some amide nitrogen to citrulline in the liver, thus giving rise to arginine. Probably, however, most of the glutamine used for urea synthesis does not involve Krebs's ornithine mechanism.

2. *Glutamine mechanism*

It now appears clear that urea can be formed in the liver by a mechanism which appears to be independent of Krebs's system and that glutamine is the immediate source of nitrogen for this synthesis. Leuthardt and Glasson (130, 131, 135, 136) and Krebs (116) have reported that fasting guinea pig liver slices, and to a less extent rat liver slices, formed urea from glutamine more rapidly than from any other substrate besides ammonium chloride. Asparagine to a lesser extent (and to a still smaller extent succinamide) can replace glutamine as a substrate in the synthesis of urea by this extract. Leuthardt and Glasson report that vitamin B_1 , its phosphate ester (136), and bicarbonate (135) catalyze urea synthesis in vitamin B_1 deficient rats.

We do not know as yet what fraction of urea synthesized *in vivo* is normally derived from glutamine and what portion is formed through operation of Krebs's cycle. Nor can we do little more than surmise, at present, what factors influence the relative amount of urea formed by either of these mechanisms.

3. *Consideration of energy relationships in urea synthesis*

As both Leuthardt (130) and Borsook (23, 24, 25) have pointed out, the process of urea formation from carbon dioxide and ammonia includes some reactions which can supply free energy to the extent of 14,000 cal. per mole of urea. Pyruvate, lactate (119), and oxalacetate (135) catalyze the reaction in slices. Leuthardt and Glasson state (134) that the presence of some autoxidizable substance, such as these substances or glucose or citric, maleic, or fumaric (not succinic) acid, is required for urea formation from ammonium chloride, but that this is not the case when glutamine, asparagine, or succinamide is the source of the nitrogen. That is, compared with the energy required for urea formation from ammonium chloride or ammonium glutamate, the energy change in the formation of urea from amide nitrogen is believed to be smaller. Nevertheless, the formation of urea from both glutamine and ammonia is prevented by cyanide

and other materials which poison oxidative, energy-giving systems. Leuthardt (130) states that the free-energy change involved in the formation of urea from glutamine and carbonic acid is less than half that involved in its formation from ammonium and bicarbonate ions. Although his calculations were based on an assumption which he would probably now change (131)—*viz.*, that pyrrolidone-carboxylic rather than glutamic acid is formed—his argument that the energy relations are such as to favor urea formation from glutamine may prove to be correct once the necessary thermodynamic data have been obtained. Leuthardt found that in the absence of glucose, 40 to 60 per cent of the amide nitrogen of glutamine was converted to urea nitrogen and he believes that, because ammonium ion does not accumulate in appreciable quantities in the liver slices, hydrolysis of glutamine to glutamic acid is not the first step in this reaction. Asparagine and the amide of succinic acid donate amide nitrogen to urea formation, but glutamic acid is only slightly active; hence it appears that the $-\text{CONH}_2$, and not the $\alpha\text{-NH}_2$, nitrogen of glutamine is most easily converted to urea nitrogen. While addition of glutamic acid to liver slices taken from starved animals increased urea formation from ammonia (though not in the presence of lactate), Leuthardt believed that this was due more to the oxidation of glutamic acid as a source of energy for urea formation than to the conversion of glutamic acid nitrogen into urea nitrogen.

4. *An evaluation of criticisms of Krebs's theory*

A number of observations once levelled as criticisms of Krebs's theory of urea formation might better be considered as evidence indicating the existence of an alternative and independent pathway of urea synthesis. These are considered below because they offer further proof of the participation of glutamine in urea synthesis. They indicate also that the capacity for urea formation of the system utilizing glutamine is probably at least equal to that of the system postulated by Krebs.

Bach (11, 13) added ornithine to the substrate of liver slices in concentrations (600 mg. per 100 cc.) that completely inhibited and possibly reversed the enzymatic hydrolysis of arginine to ornithine and urea, and noted that after a preliminary lag, urea formation proceeded. Urea synthesis from ammonium lactate was as rapid when sufficient ornithine was present to inactivate completely the liver arginase, as when but little ornithine was present. These observations, he concluded, meant that urea could be formed by a pathway other than through arginine. He failed to prove, however, that the concentration of arginine in his system did not become so high as to overcome part of the competitive inhibition produced by ornithine (9, 11, 99, 267, 292) nor did he indicate whether or not, in the meantime, the concentration of ornithine had been decreased (116) by conversion to citrulline. Later Bach *et al.* (12) showed that the concentration of ornithine decreased during incubation with liver slices.

Trowell (263) raised another objection to Krebs's theory and questioned not only the place of arginine in the cycle but also that of citrulline. He claimed that ammonia and ornithine often yielded more urea than equivalent amounts

of arginine (especially when the concentration of ornithine was so high as to inhibit arginase) and that ammonia and citrulline yielded less urea than either. This is consistent with the statement of Gornall and Hunter (79) that the speed of conversion of citrulline to arginine in liver is considerably less than that of the conversion of ornithine to citrulline. Since liver slices contain enzymes capable of synthesizing glutamine from ammonia and glutamic acid (112), the ammonia present might form urea through the glutamine cycle. Further, the catalytic effect of ornithine observed by Trowell (263) was much more prolonged than that of citrulline. He suggested that the rôle of liver arginase was to provide free ornithine and that the main path of urea formation from ammonia is not through arginine.

Furthermore, Bach (11) and Borsook and Dubnoff (23, page 195) noted that when large concentrations of citrulline were present, the amount of nitrogen in the urea formed was equal to the amount of ammonia nitrogen used, whereas from Krebs's theory one would expect only half the urea nitrogen to come from the ammonia. This would suggest that in this case urea formation took place largely through a path such as the glutamine mechanism rather than the Krebs's cycle.

Since urea is formed rapidly from ammonia when the concentration of ornithine is so high that arginase hydrolysis of arginine is almost completely inhibited (13), and since neither Borsook and Dubnoff (22) nor Krebs (116) was able to demonstrate conversion of citrulline to arginine in liver (rat and guinea pig), it seems doubtful if more than a part of the urea formed goes through citrulline to arginine. Though Leuthardt (133) and Bach (11) suggested that glutamine played a rôle in urea formation as a nitrogen and carbon dioxide carrier, Borsook (23) has been unable to observe evidence of transport of the amide group of glutamine to ornithine. The structural similarity between ornithine and glutamine should not be overlooked, and it is to be remembered that mechanisms for the conversion of ornithine to glutamic acid and glutamine are known to be present in the kidney and liver (22, 115, 206).

The fact that glutamine is used in urea synthesis in animals reminds one of the analogy made by Boussingault (26): namely, that the detoxification of ammonia in plants by the formation of amides is the functional equivalent of urea formation in animals. It is conceivable that in animals the mechanism for ammonia detoxification by formation of amides is the same as in plants, but that in animals the process goes a step further to urea or purine formation. Björkstén's evidence (18), which Chibnall considers worthless (45, page 13), that protein synthesis in wheat seedlings can occur at the expense of urea, suggests that in plants there may be an enzymatic mechanism which involves the same conversion of amide nitrogen to urea, except that in these experiments the process was observed to proceed in a direction reverse to that which we believe to be characteristic of animal metabolism. That is, in Björkstén's experiments urea nitrogen appeared to be converted into a form which was useful for protein synthesis, whereas, in some animals, nitrogen in useful forms is catabolized to urea.

The observation of Bollman, Mann, and Magath (20) that hepatectomized

animals are unable to synthesize urea is not inconsistent with the idea that one step of Krebs's cycle, *viz.*, formation of arginine from citrulline, may normally occur in the kidney as well as the liver.

Formation of arginine in the kidneys could not continue unless the liver were present to replenish the supply of citrulline to the kidney.

The observation of Mann *et al.* that the concentration of blood urea rises in nephrectomized dogs is again not inconsistent with the view that the kidney may normally play an appreciable rôle in urea synthesis. In nephrectomized dogs urea may be synthesized by any or all of three mechanisms: (1) by hydrolysis of arginine synthesized from citrulline in the liver; (2) by hydrolysis of arginine liberated from proteins (either exogenous or endogenous); and (3) by synthesis from glutamine. The conclusion that the liver is essential for urea synthesis has not been altered by more recent advances in our knowledge of the metabolic processes involved.

Reid's observation (198) that, during the first 24 hr. after bilateral nephrectomy, rats synthesize only 66–75 per cent as much urea as control animals in which both kidneys are intact was interpreted as indicating the rôle which the kidney played in deamination of amino acids. His experiment might indicate equally well a rôle played by the kidney in synthesis of arginine from citrulline. Unfortunately, this experiment gives no clue as to the ratio of the amounts of urea formed by the two (ornithine and glutamine) cycles either under these conditions or under normal conditions. Further, if the effectiveness of one of these cycles is reduced by absence of the kidneys, it is probable that the alternative cycle will be stimulated to greater activity on this account. It is more than possible that the elevated level of blood urea in the nephrectomized animal partially inhibited urea synthesis by the glutamine cycle. It would be interesting to have for comparison the rate of urea synthesis in animals in which the ureters were tied off so that the level of blood urea would rise while the kidney tissue remained *in situ* to perform deamination and/or arginine synthesis from citrulline.

The following observation of Shiple and Sherwin (240) strongly suggests that glutamine plays a most significant rôle in urea synthesis. Feeding of phenylacetic acid (α -toluic acid) to men in nitrogen equilibrium did not increase either protein catabolism or total urinary nitrogen, but decreased urine urea to 15 per cent of its usual level. The other 85 per cent of initial urea nitrogen excretion appeared as *N*- α -toluylglutamine ("glutamine phenylacetic acid") (240), $C_6H_5CH_2CONHCH(COOH)CH_2CH_2CONH_2$ (94, 259). While this does not prove that the nitrogen which goes to form urea passes through glutamine, it does indicate that the nitrogen, whatever its source, can be tied up in glutamine, and since urinary nitrogen was not increased by the feeding of phenylacetic acid, it suggests that both nitrogens of glutamine are ordinarily available for urea formation. Because normally only a small amount of glutamine is excreted in the urine, the large amount of glutamine which Shiple and Sherwin found to be coupled with phenylacetic acid appears to have been called forth as a response to the presence of phenylacetic acid and in a manner analogous to the way in which

glycine is made available for coupling when large doses of benzoic acid are given. Nevertheless, the fact that Shiple and Sherwin's subjects were in nitrogen equilibrium and that their total urinary nitrogen output did not increase in response to large doses of phenylacetic acid makes it appear significant that the large drop in urea nitrogen excretion was equalled by the nitrogen in the coupled glutamine excreted. This observation not only suggests a rôle for glutamine in urea formation but also indicates a rôle which glutamine plays in detoxification.

J. RÔLE OF GLUTAMINE IN DETOXIFICATION

Many early workers, chiefly Thierfelder and Sherwin (259, 260), have studied this in man. Ambrose, Power, and Sherwin (2) report that in man 95 per cent of ingested phenylacetic acid when given in small doses is excreted as a glutamine derivative (combined through the α -NH₂) and 5 per cent as a glucuronide. Power (186) showed the same to be true in the chimpanzee. Leuthardt (133) has claimed that guinea pig (but not rat) kidney and liver couple glutamine with benzoic to give hippuric acid⁶ (132, 134, 135), or with phenylacetic acid, but glutamic acid will not replace glutamine in this process. If this is true, Young's suggestion (309) that the reaction is limited to man and higher apes is incorrect. Sherwin *et al.* (238) showed that on feeding 15 g. of phenylacetic acid to man half the ingested amount was excreted combined with glutamic acid and more glutamine was found in the urine combined with phenylacetic acid than was present free or combined in food (239). He concluded, therefore, fifteen years before Krebs demonstrated the enzyme glutaminase, that man is capable of synthesizing glutamine, and that glutamine is capable of acting as a detoxifying agent. Species differences, however, are fairly sharp (309). Thus dogs (212), rabbits (213), cats (217), and monkeys (237) combined most of the ingested phenylacetic acid with glycine and not with glutamine, and rat liver or kidney slices failed to use glutamine in detoxification of benzoic or phenylacetic acid; fowls (262) combine the phenylacetic acid with ornithine. The glutamine, glycine, or ornithine derivatives formed by acetylation of the α -amino groups with phenylacetic acid when fed to man or any animal are excreted unchanged (241).

K. RÔLE OF GLUTAMINE IN THE KIDNEY

1. *Glutamine as precursor of urinary ammonia*

The early work on this subject has been reviewed by Schneller (221). Polonovski, Boulanger, and Bizard (184, 185) and Pitts (180) have concluded that urea is not a precursor of urinary ammonia. The writer (9) was unable to demonstrate the presence of urease in dog kidney. For urinary ammonia to be derived from urea through action of urease, dog kidney would need to contain more than two hundred times the concentration of urease which could escape detection by the procedure employed.

⁶ Leuthardt believes that benzoylglutamine (134) is probably the first product and that this is degraded to hippuric acid. Results of the experiments designed to elucidate the mechanism of this formation of hippuric acid are not conclusive.

Experiments by Van Slyke, Phillips, Hamilton, Archibald, Fitcher, and Hiller (268), on dogs in which ammonia excretion was accelerated by administration of hydrochloric acid, yielded the following results: (a) The conclusion of Nash and Benedict that the ammonia concentration is greater in the renal venous blood than in arterial blood was confirmed; hence the ammonia excreted in the urine is not derived from preformed blood ammonia, but must be formed in the kidneys from some other nitrogenous material. (b) All the urea removed from the blood by the kidneys was excreted unchanged in the urine; hence urea could not serve as a significant source of the urinary ammonia. (c) The total amount of α -amino acid nitrogen extracted by the kidneys from the blood plasma was calculated from analyses of arterial and renal venous blood plasma by the ninhydrin carbon dioxide method, together with measurements of renal blood flow, and was found to be either nil or too little to provide ammonia at the rate excreted in the urine. (d) The amide nitrogen of glutamine, determined by Hamilton's (86, 87) application of the ninhydrin carbon dioxide method and by the author's (6, 7) enzymatic method, was much less in the plasma of renal venous blood than in the plasma of arterial blood. The difference, multiplied by the rate of plasma flow through the kidneys, indicated that the kidneys removed glutamine amide nitrogen from the blood at a rate sufficient to provide 60 per cent or more of the total ammonia passed by the kidneys into the urine and into the renal venous blood. It therefore appears that glutamine is the main precursor of urinary ammonia in the dog, although a smaller portion may be derived from deamination of α -amino acids.

In studies of the mechanism by which the ammonia is formed in the kidneys, the writer (7) examined the kidneys of dogs for glutaminase, capable of splitting ammonia from the acid amide group of glutamine, and consistently found this enzyme, as had Krebs (112) in the kidneys of other species. Glutaminase from a dog kidney extract was then added to dog plasma and ammonia was produced. That did not mean that glutaminase was necessarily the source of this ammonia. However, these findings indicated that: (1) blood contained something which liberated ammonia in the presence of kidney extract, and (2) kidney contained an enzyme capable of liberating ammonia from something present in plasma as well as from added glutamine. It seemed likely that glutamine was the substrate present in plasma. After an exhaustive study of the specificity of the glutaminase method it was concluded (6, 7, 268) that the difference in concentration of glutamine amide nitrogen in the arterial and renal venous plasma was sufficient to account for 60 per cent or more of the ammonia in urine of dogs in acidosis.

Whether or not α -amino acids, as suggested by Krebs (110), or amides of blood proteins (19), or glutamine in the red cells is the source of the remaining 40 per cent of urinary ammonia remains undetermined. There may be species differences in the proportion of urinary ammonia that is derived from glutamine. That the proportion of urinary ammonia derived from glutamine may be less in man than in the dog is suggested by the observation that normal human kidneys have a much lower concentration of glutaminase than dog kidneys (6, 9).

Wassermeyer (296) suggested erythrocyte adenylic acid as a source of urinary

ammonia. However, spectrophotometric studies of dialysates of renal venous and arterial plasma of dogs in acidosis (author's observations (9, 268)) failed to indicate either deamination or removal from plasma by the kidney of appreciable amounts of adenosine derivatives. The peak of the characteristic absorption curve of adenosine and of its phosphate derivatives is at a wave length of 260 $m\mu$. The absorption of light of this wave length by the above-mentioned dialysates was found to be equivalent to an adenosine + adenosine phosphate concentration in plasma of $4 \times 10^{-5} M$. This is equivalent to a concentration of 2.4 mg. of adenylic acid per 100 cc. plasma. On deamination this would yield 0.1 mg. of ammonia nitrogen per 100 cc. of plasma (7). However, other constituents of plasma dialysates absorb light of this wave length; therefore the plasma concentration of adenylic acid is probably much less than is indicated by this maximal figure. For the urinary ammonia to be derived entirely from plasma adenylic acid, in the experiments quoted on acidotic dogs (268), more than twice this maximal amount of adenosine or adenylic acid would need to be present in the plasma of the renal artery and all would have to be utilized by the kidney, whereas, actually, less than 10 per cent was extracted from the plasma or deaminated as the blood passed through the kidney. It was concluded, therefore, that less than 5 per cent of the urinary ammonia could arise from deamination of plasma adenosine or its derivatives.

The value of 17.8 mg. of adenine nucleotide per 100 cc. given by Buell and Perkins (41) was for human whole blood; the nucleotide is practically all in the cells.

Whether or not adenosine and its derivatives serve as an intermediary carrier of nitrogen from glutamine to urinary ammonia or whether the relatively large reserve of adenosine in the red blood cells is used directly to form part of the urinary ammonia is still undetermined, but glutamine appears to be the plasma precursor of most of the urinary ammonia. Kleinzeller (108) has so far been unsuccessful in causing enzymatic synthesis of adenosine di- or tri-phosphate from inosine di- or tri-phosphate in the presence of adenosine triphosphate deaminase, glutamic acid, glutamine, asparagine, or ornithine. However, he assumes that the synthesis occurs *in vivo*.

2. Presence of glutaminase in human kidney

Examination of glutaminase and purine nucleoside deaminase of kidneys from fifty-two different humans showed (7, 9) that glutaminase is present also in the human. It was not found in the kidneys of cases which had had severe kidney involvement, such as chronic hemorrhagic nephritis or arteriosclerotic changes. These cases had, however, considerable purine nucleoside deaminase activity. Since it is known that the capacity to form urinary ammonia is largely lost early in kidney disease⁷, the persistence of the activity of the nucleoside deaminase and the loss of glutaminase points to glutamine being the ultimate

⁷ In diabetic coma, however, the urea clearance may be temporarily reduced to 5 per cent of normal, the plasma urea may rise to 150 mg. per 100 cc., and the urinary ammonia production remains unimpaired, as shown by McCance and Lawrence (150).

source of urinary ammonia rather than nucleosides or precursors thereof. While the author has had no difficulty in demonstrating the presence of glutaminase in human kidney, it is usually present in concentrations 1/10 to 1/100 those found in dog kidney (7). However, it has been found in kidneys of premature infants and of patients seventy-seven years old. Oddly enough, ten of the eighteen kidneys showing unusually high glutaminase activity have come from patients who had new growths of lung, prostate, stomach, intestine, or brain, usually without visible metastatic involvement of the kidney. The increased concentration of glutaminase in these kidneys may have been a response to increased breakdown of tissue rather than a specific response of the host to a new growth.

The fact that Marples and Lippard (145), McCance and Widdowson (151), and Branning (30) report that human premature infants are peculiarly susceptible to acidosis, indeed are constantly on the threshold of acidosis, and that the author found that kidneys of premature infants contained less glutaminase per gram than those of full-term infants, suggests that the relative lack of kidney glutaminase to provide ammonia may contribute to their acidosis. Inability of prematures to form adequate ammonia to prevent loss of fixed base in the urine may, however, not be the whole cause of their acidosis (30, 145).

3. *Glutamine and citric acid metabolism*

Hunter and Leloir (102) have demonstrated that citric acid formation from acetoacetic acid in the presence of oxaloacetate by kidney cortex is increased several-fold by the addition of glutamate or α -ketoglutarate. Glutamine as a precursor and reservoir of these two catalysts can be considered, in this sense, as playing a rôle in the formation of citric acid. From the work of Hamilton (87) it would appear likely that a large part of the cofactor which Hunter and Leloir found in muscle extract was free glutamine. This glutamine would be moderately stable under the heating conditions employed and would be hydrolyzed to glutamic acid by the glutaminase in the dog kidney (7) preparation of citrogenase.

L. RÔLE OF GLUTAMINE IN MISCELLANEOUS SYSTEMS

1. *Relation of glutamine to reamination of nucleotides or nucleosides*

There may prove to be a close tie-up between the reversible glutaminase system and nucleotide or purine nucleoside deaminase systems. Evidence at hand does not warrant a detailed discussion of this point, but suffice it to say in suggestion that (1) cozymase has been shown to be a necessary factor in the transamination of glutamic acid *in vitro*³, and (2) Bruhl (39) observed that in convulsive states the concentration of ammonia in brain tissue increases as that of adenylic acid decreases. Riebeling (200) states that 75 per cent of the am-

³ Glutamic acid dehydrogenase of higher plants has been said to act only in presence of coenzyme I, while that of lower plants, such as yeast or *B. coli*, acts only in the presence of coenzyme II, and that of animal tissues acts in the presence of either coenzyme I or coenzyme II (63).

monia which brain tissue can liberate comes from adenylic acid, and Weil-Malherbe (300) drew attention to the unique rôle played by glutamic acid in brain metabolism. The work of Sapirstein and the clinical work of Price, Waelsch, and Putnam would seem to favor the conclusion that ammonia formation and detoxification normally go hand in hand. That reamination of inosinic acid takes place as a result of action of enzyme systems which utilize the amide of glutamine is an attractive hypothesis, but so far the process has not been demonstrated (113). It is of interest in this connection that striated muscle (especially heart and diaphragm) is rich in both glutamine and adenosine or its products.

TABLE 1

Distribution of glutamine in animal tissues

(Milligrams of glutamine amide nitrogen per 100 g. of tissue (wet weight))

TISSUE	NINHY- DRIN CARBON DIOXIDE METHOD*	ACID HYDROLYSIS METHOD†						
		Dog	Dog	Cat	Rabbit	Pigeon	Horse	Marmot
Heart.....	21.6	20.3	19.0	10.5	6.9	21.2	23.2	9.4
Brain.....	6.1	11.0	11.0	8.5	10.0			
Liver.....	4.3	8.5	8.0	7.0	5.0			
Skeletal muscle....	11.7	10.8	8.3	4.9				
Kidney.....	1.1	4.5	4.3	2.5				
Spleen.....	5.9							
Uterus.....	2.3							
Lung.....	1.9							
Stomach.....	3.1							
Small intestine....	5.3							
Large intestine....	4.8							

* Figures reported by Hamilton (87).

† Figures reported by Ferdman *et al.* (69).

2. Presence of free glutamine in animal tissues

Ferdman *et al.* (69) studied the glutamine amide nitrogen in several organs. Because his results are not elsewhere available in English, they are reproduced in table 1. The concentration of glutamine in cats and marmots decreased in starvation and during hibernation. Hamilton (87), using the more specific ninhydrin carbon dioxide method, has obtained glutamine values for dog tissues which confirm in general those given by Ferdman. In view of the low concentration of glutamine in dog kidney it is of interest to note that Krebs (113) found that the kidneys of the dog, pig, cat, and pigeon (unlike those of the guinea pig, rabbit, sheep, and rat) do not synthesize glutamine. According to Krebs (113), certain herbivores (such as rabbits and guinea pigs, the kidneys of which synthesize glutamine rapidly) store considerable glutamine in their kidneys. Ferdman's single value for rabbit kidney does not seem to agree with Krebs's finding.

Glutamine has been isolated from three different tissues and animal species by three laboratories. McIlwain *et al.* (153) in 1939 isolated it from horse meat, following a preliminary precipitation with phosphotungstic acid which separated glutamine from 97.6 per cent of the nitrogenous impurities present in meat at the expense of a 60 per cent loss of glutamine. Örström *et al.* (170) in the same year reported the isolation of glutamine synthesized in pigeon, fowl, and duck livers, employing precipitation by mercuric nitrate and obtaining a 60 per cent yield of glutamine. Frenkel (69) isolated glutamine from horse brain after a precipitation with mercuric and silver ions by a procedure which gave a 30 per cent yield.

3. *Effect of glutamine on carbohydrate metabolism*

A relation of glutamine and its nitrogen metabolism to carbohydrate metabolism in animals is indicated by the observation of Örström *et al.* (170) that pigeon, fowl, and duck livers form glutamine from ammonium pyruvate at the rate of 30 mg. per hour per gram of dry tissue. It is probable that α -ketoglutarate is an intermediate when this process takes place in the kidneys of guinea pigs, since glutamine formation here is more rapid from ketoglutarate than from pyruvate. However, in liver slices of pigeon, fowl, and duck, glutamine synthesis was found to be more rapid with pyruvate than with ketoglutarate. Amide formation in rat liver was slower and was not accelerated by the addition of ammonium pyruvate. In the cases of mammalian brain and retina, and guinea pig and rabbit kidney, glutamine synthesis from ammonium glutamate was five to ten times as rapid as from ammonium pyruvate.

Further indication that glutamine is involved in carbohydrate metabolism is to be found in the observation that glutamine and glutamic acid show striking effects on carbohydrate oxidation and on lactic acid production in those tissues which contain glutaminase (298). In this connection Smythe's observation (245) that ammonia and amides of dicarboxylic acids increase the rate of fermentation by yeast should be noted. Tatum (256) noted that glutamine was necessary for the fermentation of starch by butyric acid bacteria. Harris (90) observed a marked drop in plasma and spinal fluid glutamine level following administration of insulin.

4. *Effect of glutamine on purine metabolism*

Örström, Örström, and Krebs (169) state that glutamine and oxalacetate stimulate the formation of hypoxanthine in pigeon liver and the formation of uric acid in fowl, duck, rat, and guinea pig livers which contain xanthine oxidase.

M. PHYSIOLOGICAL RÔLES OF GLUTAMINE IN MAMMALS

The finding that in acidotic dogs glutamine is the source of a large part of the urinary ammonia (268) provides the first demonstrated example of a physiological function for the glutamine-glutaminase system in the animal kingdom.

The earlier finding of Shiple, Sherwin, *et al.* (page 194) that phenylacetic acid is excreted combined with glutamine at the expense of urinary urea showed that glutamine was capable of acting as a detoxifying agent in certain species,

but the identity of the naturally occurring metabolites detoxified by combination with glutamine remains unknown.⁹ Indeed, by stopping the normal conversion of glutamine to urea they may have done more to provide circumstantial evidence of a mechanism of urea formation than could be appreciated at the time. Nevertheless, the statement of Örström, Örström, Krebs, and Eggleston (170) is almost as true today as when it was written: "The significance of the synthesis of glutamine remains obscure as long as the rôle of glutamine in tissue metabolism is unknown. (There is) . . . no doubt that glutamine is a factor of general importance in cell metabolism but the nature of its function is not yet clear."

However, it seems legitimate to speculate that glutamine performs some of the functions in animals that it has been shown to perform in plants, such as neutral transport and storage of labile $-\text{NH}_2$ for protein synthesis. This synthesis could involve either incorporation of glutamine, as such, into the protein molecule, or of glutamic acid, or preliminary formation of other amino acids by transfer of nitrogen from glutamine to α -keto acids arising from metabolism of carbohydrate, fat, or other amino acids. The observation of Schoenheimer *et al.* (222) that isotopic nitrogen, when fed to animals as ammonia or amino acid, was recovered to a large extent as amide nitrogen of tissue proteins, supports the idea that in animals there is a rapid exchange between amide groups in proteins and ammonia or amino or amide groups of free acids. The earlier observations of Bliss (19) likewise support this view. Nitrogen stored as amide is at a higher energy level than if it were present as ammonia.

In animals (at least in omnivora and carnivora) it would at present appear that glutamine plays a more dominant rôle than asparagine, whereas in most plants asparagine is the more abundant amide.

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⁹ Although phenylacetic acid is said to result from the oxidation of phenylbutyric acid by the kidneys of calf, sheep, and dog (245a), it appears unlikely that phenylbutyric acid occurs naturally in any significant concentration.

However, the odor of some specimens of old, dried, human urine cannot be distinguished from that of phenylacetic acid (α -toluic acid). It may be, therefore, that "glutamine phenylacetic acid" (*N*- α -toluylglutamine) or the corresponding 4-hydroxy compound occurs naturally in human urine and that phenylacetic acid or 4-hydroxyphenylacetic acid is derived from tyramine, which in turn would be derived from tyrosine by decarboxylation (94a). Amine oxidation of tyramine and β -phenylethylamine has been demonstrated by Bernheim and Bernheim (15a), using heart and liver slices. After feeding tyramine to dogs, 25 per cent was recovered from the urine as *p*-hydroxyphenylacetic acid (65a). A 70 per cent conversion to this acid has been observed on perfusion of cat and rabbit liver with tyramine. Action of another amine oxidase on the related compound mescaline also yields the corresponding acid (15b) in dog and rabbit but not in man (242a). The acid is then excreted in urine. It may be, therefore, that oxidation products of tyrosine provide the naturally occurring substrate for the reaction observed by Shipley and Sherwin *et al.* in humans.

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CARBONYL BRIDGE COMPOUNDS AND RELATED SUBSTANCES¹

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CONTENTS

I. Introduction.....	209
II. Nomenclature.....	209
III. Types of bridged compounds.....	211
A. Saturated rings.....	211
B. Unsaturated rings.....	212
1. Rings having more than six carbon atoms.....	212
2. Six-membered rings	215
(a) Methods of preparation	215
(b) Properties of six-membered unsaturated rings ..	217
(i) Bicyclic carbonyl bridge compounds.....	217
(ii) Multicyclic carbonyl bridge compounds ..	223
(a) The polynuclear series	223
(b) The indenenes and related substances ..	224
(c) Other types of addends ..	245
(d) Unsaturated six-membered rings having lactone and anhydride bridges..	249
IV. Loss of the bridge and its relation to the double-bond rule ..	251
V. Carbinol bridge compounds ..	259
VI. References.....	266

I. INTRODUCTION

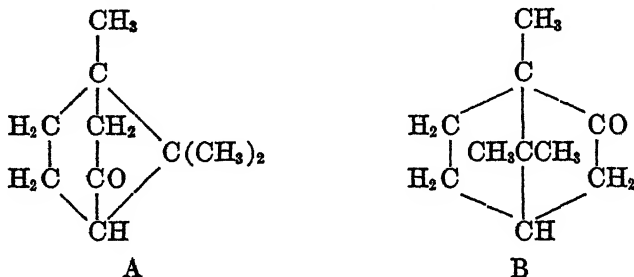
Alicyclic compounds containing a group of atoms not connected to adjacent atoms in a carbon ring have long been known to organic chemists, but until the advent of the diene synthesis the available varieties were limited almost entirely to the terpenes and their derivatives. During the last decade there has been considerable interest in such bridged compounds, especially in those in which a carbonyl group serves as the bridge; for convenience, these compounds have been called "carbonyl bridge compounds." They have all contained at least one ethylenic linkage and have been polyarylated. Their most characteristic behavior has been the elimination of the bridge when heated. The known carbonyl bridge compounds of this type and related substances, as well as a few molecules containing carbinol, lactone, and anhydride bridges, which show a similar behavior in certain reactions, will be described in this paper. The terpene ketones, which can be visualized (formula A) as containing a $\text{—CH}_2\text{CO—}$ bridge, will not be considered, since none of their properties appear to be connected with the presence of the bridge.

II. NOMENCLATURE

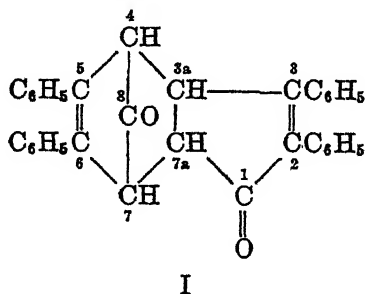
To the organic chemist, the term "bridged" implies a condensed cyclic system having three or more atoms (usually carbon) common to two rings. Bridged

¹ Communication No. 1033 from the Kodak Research Laboratories.

rings have long been known in considerable variety, and are commonly associated with terpene derivatives in the camphor series and with the tropine alkaloids, though many other systems are recorded in the literature. There is nothing in this nomenclature to suggest anything in regard to the nature of the bridge, and examples are known in which the atoms forming the bridge are carbon, nitrogen, oxygen, etc., alone or in combination. The term "bridged," therefore, is used for convenience. Through continued usage, this term has come to mean any group of atoms in a cyclic substance connecting two other atoms that are not in adjacent positions, and by convention the shortest chain is considered to be the bridge. Thus, camphor is ordinarily written as B rather than A. In modern



nomenclature, the bridge is indicated by placing numerals in brackets. The numerals indicate the number of atoms in the chains between the bridgeheads; in *Chemical Abstracts* usage, the bridge is the last of the bracketed numerals. Thus, camphor is 1,7,7-trimethylbicyclo[2.2.1]-2-heptanone. In polycyclic ring systems having more than two rings, the bridge is inserted in the name, as methano, ethano, etc. Thus, the substance shown in formula I is named 2,3,5,6-tetraphenyl-3a,4,7,7a-tetrahydro-4,7-methanoindene-1,8-dione.



When one examines three-dimensional models, a better idea of the spatial positions of the atoms comprising the ring structures is obtained. If the observer looks directly down upon the molecule, at the point of union of the bridge and ring, i.e., along the 1,4-axis, he sees three bonds radiating outwards (figure 1) at equal angles; if viewed from the side, the molecule resembles a cage (figure 2). There is no great difference in linking or spatial characteristics between the bridge and other parts of the ring. This lack of difference emphasizes the danger of distinguishing between the behavior of the bridge and that of any other portion

of the molecule. Chemical behavior, then, must be associated with the nature and type of linkage of the groups present, rather than with a peculiarity of the bridge. With this concept in mind, plane bridged formulas are used in the examples given in this paper.

III. TYPES OF BRIDGED COMPOUNDS

The bridged compounds to be described comprise those in which the ring is numerically saturated and those having an ethylenic linkage. They are further subdivided according to the size of the ring.

A. SATURATED RINGS

There are recorded in the literature two substances with the molecular formula $C_7H_{10}O$ which the respective authors represented as having a saturated six-membered carbon ring and a carbonyl bridge.

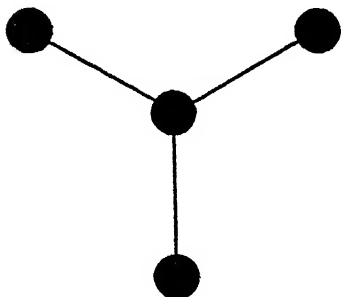


FIG. 1

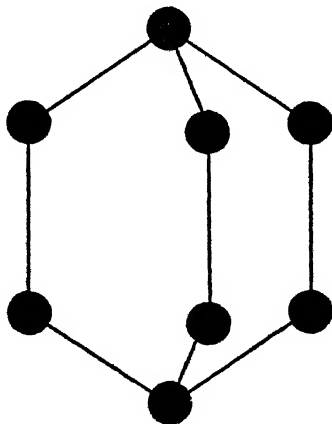
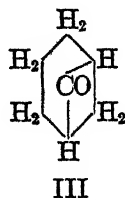
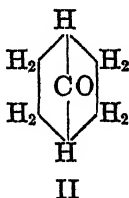


FIG. 2

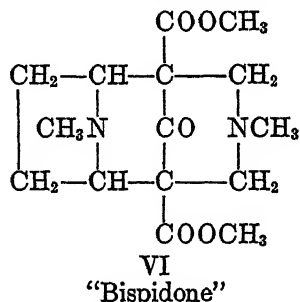
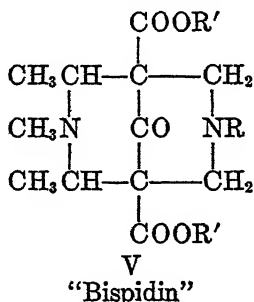
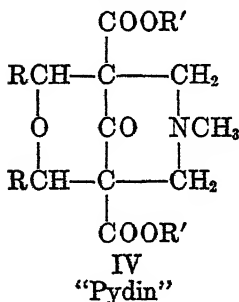
Zelinski (86) hoped to prepare norcamphane by the reduction of the ketone II, which he expected to secure by distillation of the alkaline earth salts of *trans*-hexahydroterephthalic acid. He obtained along with a small amount of benzene a much smaller fraction (b.p. 150–180°C.); from the latter fraction he prepared a semicarbazone which, he wrote, "must correspond to the ketone" (II). It was not investigated further and its existence should be considered doubtful.

By a similar procedure, Stark (75) attempted to secure the ketone III from



hexahydroisophthalic acid. He obtained a small fraction, from which was prepared a semicarbazone, m.p. 179–180°C. This ketone is referred to by Böeseken and Peek (29), who wrote, "...the ketone of Stark has the properties of an unsaturated substance which could be a cyclobutanone. It is very probable that it is, in fact, a derivative of cyclobutanone, stabilized by the other ring, placed..." However, Ruzicka and Trebler (73) pointed out that Stark could not have had the compound claimed, for its refractive index is much too high and its behavior towards bromine is like that of other unsaturated ketones. The melting point of the semicarbazone is the same as that of the semicarbazone of 1-methyl-3-cyclohexen-2-one. Thus, it appears that there is a reasonable doubt as to the correctness of the structures of both II and III.

Mannich (67–69) has described two bicyclic eight-membered heterocyclic ring systems "Pydin" (IV) and "Bispidin" (V), and the tricyclic "Bispidone" (VI). From the method of preparation they were expected to contain a carbonyl group but its presence could not be demonstrated directly; upon degradation of one of the compounds, the formation of a known pyrrolidone was taken as evidence that the carbonyl group was present as such in the bicyclic substance.

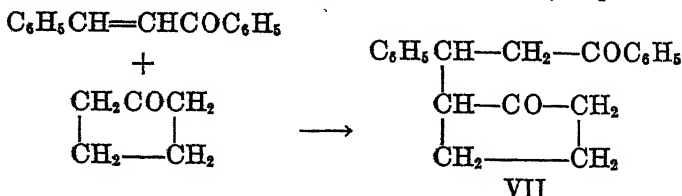


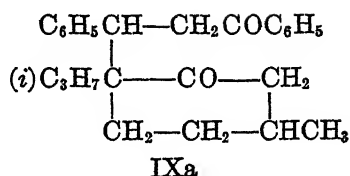
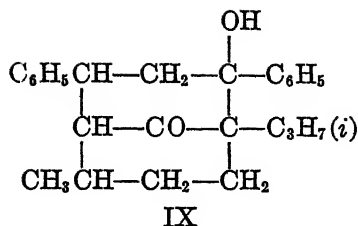
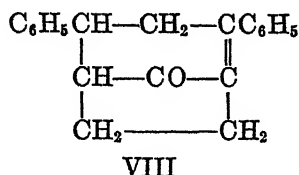
An instance of a saturated eight-membered carbon ring having a carbonyl bridge (IX) is described later because of its relation to certain unsaturated systems.

B. UNSATURATED RINGS

1. Rings having more than six carbon atoms

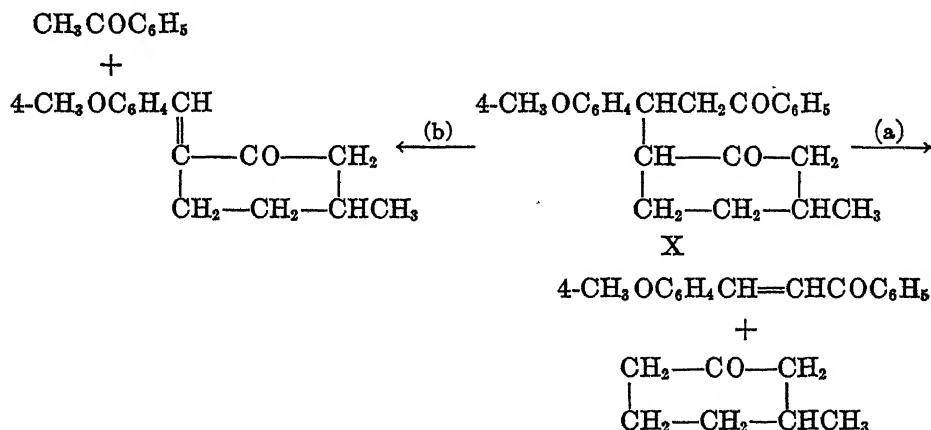
There are only a few bicyclic ring systems known having more than six atoms in the ring and containing both a carbonyl bridge and an ethylenic linkage. Half of these were prepared by Stobbe (77–83) during his investigations on semicyclic ketones, and the rest by Allen and Sallans (14). For example, cyclopentanone was added to benzalacetophenone, and the intermediate ketone (VII) thus formed was cyclized by the action of hydrogen chloride in warm alcohol to give the unsaturated bridged substance VIII, m.p. 122°C. (79).





A cyclic keto alcohol (IX) could be isolated only when menthone was selected as the addend (82). Such keto alcohols are isomeric with the corresponding addition products, and are probably intermediate products in the formation of the bicyclic ring systems. Accordingly, Stobbe took great care in establishing the open structures.

These intermediate ketones (called semicyclic ketones by Stobbe, for convenience) dissociate into their components on distillation. This cleavage may take place in two ways: for example, 2-phenacylobenzylcyclopentanone (VII) re-forms cyclopentanone and benzalacetophenone, while with 2-phenacyloanisyl-5-methylcyclohexanone (X) the products are (a) anisalacetophenone and 3-methylcyclohexanone, and (b) acetophenone and anisal-3-methylcyclohexanone (77).

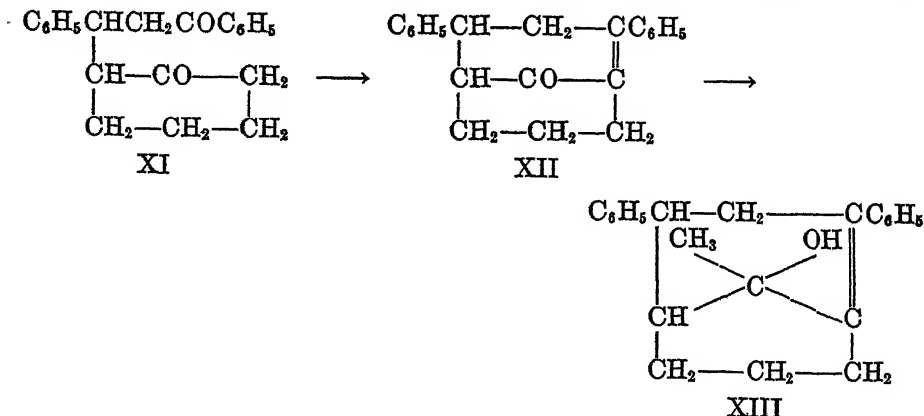


These intermediate ketones form mono- and di-oximes, oxime-semicarbazones, and disemicarbazones. The ketones can be regenerated from the disemicarbazones. The monoximes can be cyclized into heterocyclic bases, tetrahydroquinolines, or pyrhydrindenes (81). These ketones can be converted into pyrylium salts (14). They add quantitatively two equivalents of methylmag-

nesium iodide without evolution of gas (14).² They react with aromatic aldehydes, indicating the presence of a methylene group adjacent to the carbonyl group, although a dibenzal type never results (77). The location of the methyl group in X was shown by the formation of β -methyladipic acid upon oxidation (77).

The keto alcohol IX (82), however, forms only a monoxime, which does not give a heterocyclic base, and a monosemicarbazone. It can be distilled unchanged, it does not give pyrylium salts, and it does not react with aromatic aldehydes. When treated quantitatively with methylmagnesium iodide, it reacts with two equivalents, forming one equivalent of methane, a reaction which indicates one active hydrogen atom (hydroxyl). This last reaction also affords confirmation of the structure IX, for Stobbe was unable to prove the presence of the hydroxyl group. If the menthone had added to the benzalacetophenone in the opposite possible manner (IXa), an open-chain ketone would have resulted or, if a keto alcohol had been formed, it would have lost a molecule of water, as occurs with the other 1,5-diketones when they are treated with hydrogen chloride.

The bicyclic bridged ketones are obtained by warming absolute alcoholic solutions of the open-chain ketones and hydrogen chloride; a molecule of water is eliminated. Although two stereoisomers are produced, only one is obtained in quantity. The new ketones can be distilled without decomposition. They form monoximes and monosemicarbazones. They react with one equivalent of methylmagnesium iodide, forming carbinols (14). They do not cyclize to heterocyclic bases. In illustration, 2-phenacylobenzylcyclohexanone (XI)



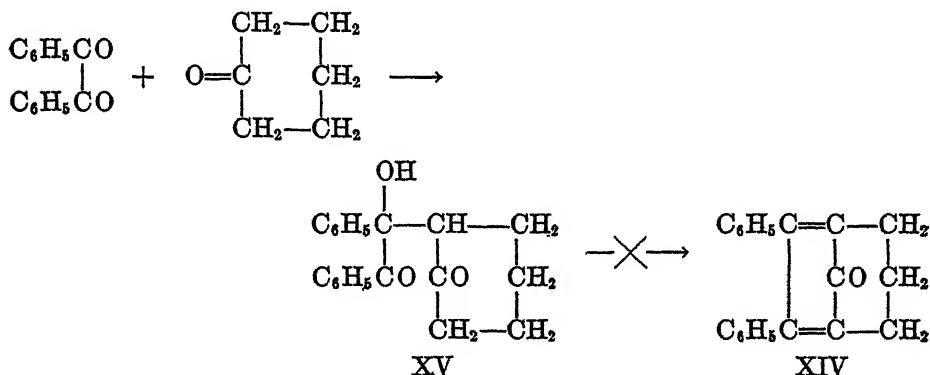
gives two stereoisomeric bicyclo[3.3.1]-2,4-diphenyl-9-keto-4-nonenes (XII) (m.p. 143°C., 151°C.), and, after treatment with methylmagnesium iodide, bicyclo[3.3.1]-2,4-diphenyl-9-methyl-9-hydroxy-4-nonene (XIII). The cyclic

² One of the modern methods of establishing the presence of a carbonyl group is to examine its behavior in the Kohler-Richtmyer apparatus (designed to measure quantitatively the amount of methylmagnesium iodide consumed and gas evolved) (66). The reagent used up, but not accounted for by the gas formed, is interpreted as having reacted by addition.

ketone XII does not decolorize permanganate or bromine, whereas the lower homolog VIII "easily adds bromine" (77) but, since bromine evolves hydrogen bromide and forms tars with all these substances containing a reduced ring, it is not a useful reagent. The arylhydrazines likewise form tars with these intermediate ketones.

The location of the double bond has not been absolutely proved. As always written by Stobbe, it is at a bridgehead and so contrary to Bredt's rule (28). The available evidence rests (a) on the method of formation (position of groups for an aldol-type reaction), (b) on the fact that the keto alcohol IX, obtained from menthone, does not lose water, and (c) on the observation that the ketone from cyclohexanone does not decolorize permanganate or bromine, as would be anticipated if the double bond were in any other position. At the moment, these substances constitute exceptions to Bredt's rule.

At this point it may be mentioned that attempts to prepare the doubly unsaturated carbonyl bridge compound XIV were unsuccessful (4). Only the first step of the reaction, analogous to the one found applicable by Japp to many aliphatic ketones (60, 61), took place to give XV. Efforts to push the reaction led mainly to the formation of resinous materials.



In summary, the few known seven- and eight-membered unsaturated bicyclic ring systems having a carbonyl bridge do not exhibit any unusual properties and they are not altered by being heated.

2. Six-membered rings

(a) Methods of preparation

There are a large number of six-membered multicyclic ring-systems having a carbonyl bridge. Nearly all have been made available by the diene synthesis (70). A few, although long known by empirical formulas, have had their structures elucidated only during the last decade. The reaction consists of the 1,4-addition of a substance having a sufficiently active ethylenic linkage to the ends of the carbon-carbon conjugated system of double bonds in a cyclopentadienone.

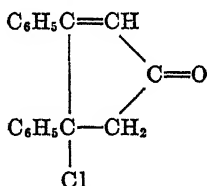
alkaline reagents on various diphenylchlorocyclopentenones (13, 16);³ the latter can be conveniently considered as related to anhydroacetonebenzil by the replacement of a hydroxyl group by a chlorine atom, so that removal of hydrogen and chlorine leaves diphenylcyclopentadienone (XVII), which at once dimerizes.

(b) Properties of six-membered unsaturated rings

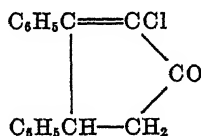
The most conspicuous property of the carbonyl bridge compounds having a six-membered ring and one ethylenic linkage is their behavior when heated—the bridge is split out as carbon monoxide. This "decarbonylation," which is usually quantitative, is rapid at 200–220°C., and in favorable instances can be detected at 75–80°C. The other product of the reaction is usually a dihydrobenzene derivative, but occasionally this is dehydrogenated to the corresponding aromatic compound; these reactions are used as a part of the proof of structure. Aromatic compounds are produced directly when acetylenic addends are used; this variation will be considered separately.

(i) *Bicyclic carbonyl bridge compounds*: Styrene adds readily to tetracyclone in boiling benzene to give the carbonyl bridge compound XXII (20). The presence of the carbonyl group is shown by the formation of carbinols when the substance is treated with Grignard reagents. When the product is heated to 200°C., carbon monoxide is rapidly given off, and the dihydrobenzene XXIII is formed;

* With many acid chlorides, anhydroacetonebenzil gives the chloride XVIII (16), for which the structure has been established to the satisfaction of those interested in the field, but with hydrogen chloride under certain conditions, both this chloride and anhydroacetonebenzil give the same isomeric chloride (16, 60), the structure of which is not yet agreed upon. The English investigators (30, 31, 32) prefer the structure XIX for this isomer.

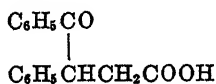


XVIII



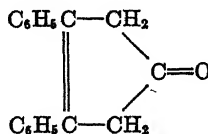
XIX

Neither group has been able to repeat the other's work *in toto*. It is the author's opinion that the assignment of any structures in such a system where anionotropic and prototropic shifts are possible should be considered as tentative and subject to revision. Ozone does not appear to be a reliable reagent, for the reference compound desylacetic acid (XX) can be obtained from 3,4-diphenyl-3-cyclopenten-1-one (XXI) where it would be least expected (31).



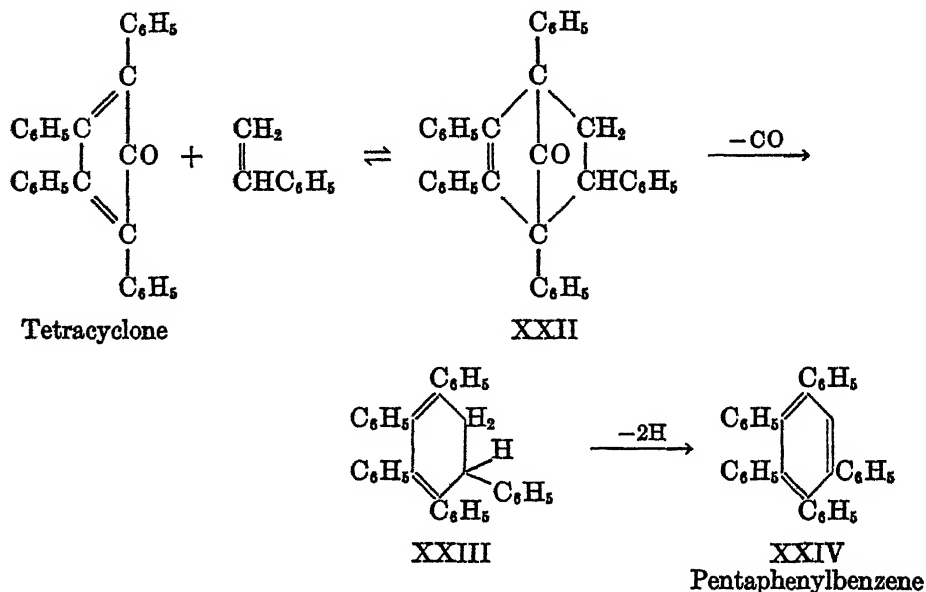
XX

Desylacetic acid

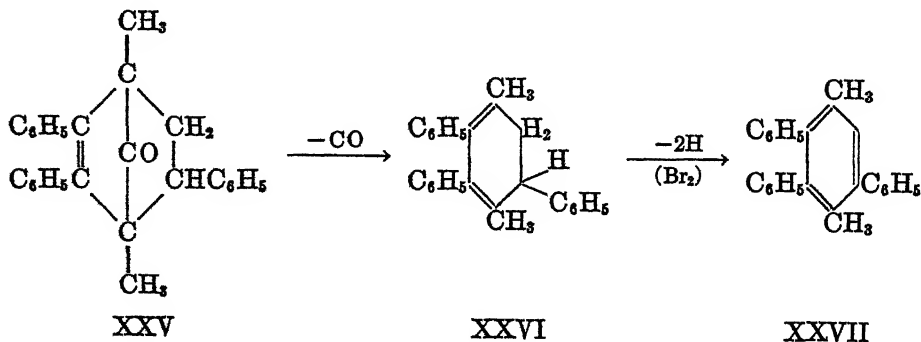


XXI

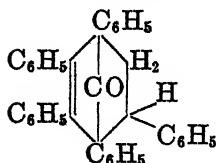
this same product is also obtained (1) when styrene and tetracyclone are refluxed together in the absence of a solvent—that is, the temperature of reaction is above that at which the bridged ketone is stable. The dihydrobenzene XXIII can be dehydrogenated to pentaphenylbenzene (XXIV) by heating with sulfur (20) or with selenium at 180–200°C. (1). It may be concluded, therefore, that the addition product XXII contained a six-membered carbon ring. A parallel



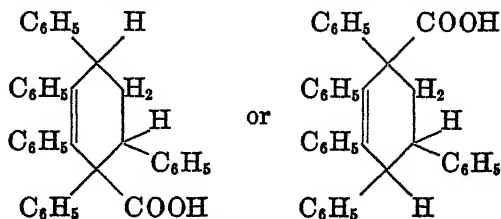
proof of structure has been used in many other instances; for example, the dimethyl analog (XXV) exhibits the same behavior, being decarbonylated to the dihydrobenzene (XXVI) and the latter being dehydrogenated by bromine to the aromatic hydrocarbon (XXVII). With this bridged ketone, the presence of the carbonyl group was detected by the formation of a 2,4-dinitrophenylhydrazone, as well as through use of the Grignard reagent (19). In all cases, the structures of the aromatic compounds have been proved by independent syntheses.



Another type of reaction, the limits of which are not yet known, is cleavage of the bridge at one end only by means of alcoholic alkaline solutions (9, 22, 23). Upon analysis of the product of the reaction, it is found that a molecule of water has been added. The new substance is a carboxylic acid, i.e., the carbonyl group of the ketone has become converted to a carboxyl group. Since the bridge may open at either end, isomeric acids are possible (the structures due to stereoisomerism are disregarded); one usually predominates.⁴ In the case of the pentaphenylated ketone XXII, the acids would be those shown in XXVIII. It is not possible to assign more definite configurations from the available facts.



XXII

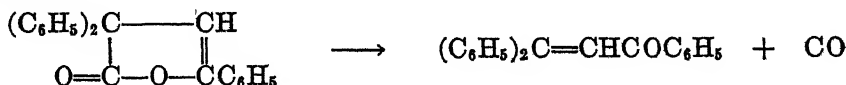


XXVIII

Upon aromatization of the acid by prolonged boiling with permanganate, pentaphenylbenzene (XXIV) is produced, but when the acid XXVIII is decarboxylated by heating at 300°C., the dihydropentaphenylbenzene XXIII is obtained (23). Thus, the presence of a six-membered ring in the addition product has again been demonstrated.

It is not possible to state whether the acid loses carbon dioxide as the result of a decarboxylation during the permanganate treatment, or whether the carboxyl group comes off as formic acid, which is then oxidized; the nature of the products obtained with more complicated substances (see pages 234, 240) indicates that the second possibility merits serious consideration.

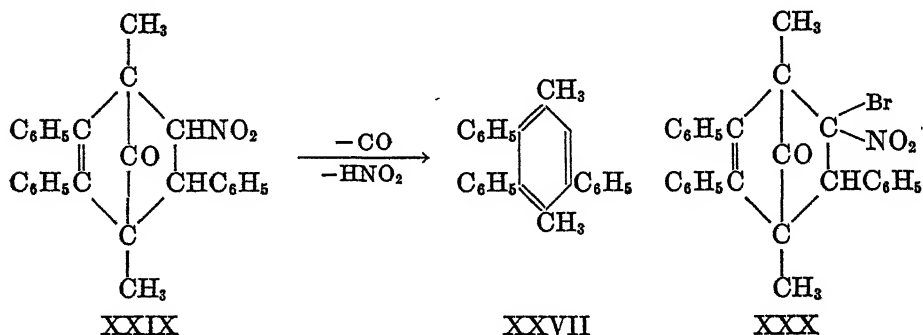
The loss of carbon monoxide, resulting from the heating of organic compounds, is not too common a reaction, especially when it takes place at moderate temperatures, and is not a pyrolysis. The carbonyl bridge compounds evolve gas most rapidly at 180–220°C., but the same process takes place very slowly at 130°C. (15) or at even lower temperatures, in very favorable instances. In most of the other instances recorded, the substance heated contains more than one oxygen atom, and the products are carbon monoxide and an aldehyde or ketone (25, 26). For example, triphenylcrotonolactones lose carbon monoxide and form α,β -unsaturated ketones (27, 61), as is illustrated by the formation of β -phenylbenzalacetophenone.⁵



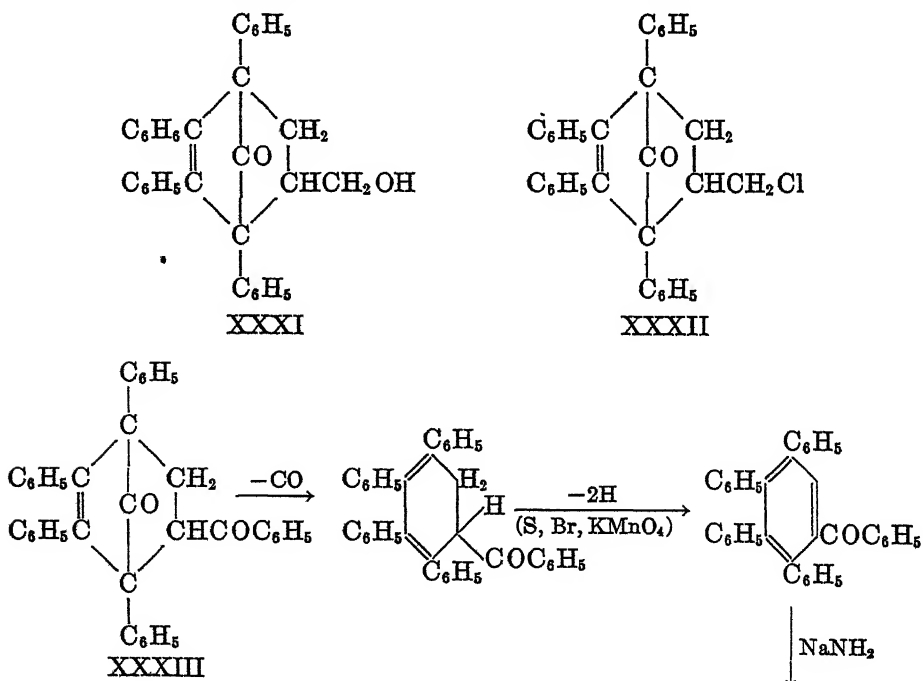
⁴ The action of alkaline reagents on carbonyl bridge compounds has not been completely investigated. Although acidic substances appear to predominate, there are usually neutral products, the nature of which has not yet been determined (23). The latter are more numerous when the carbonyl bridge compound contains other functional groups.

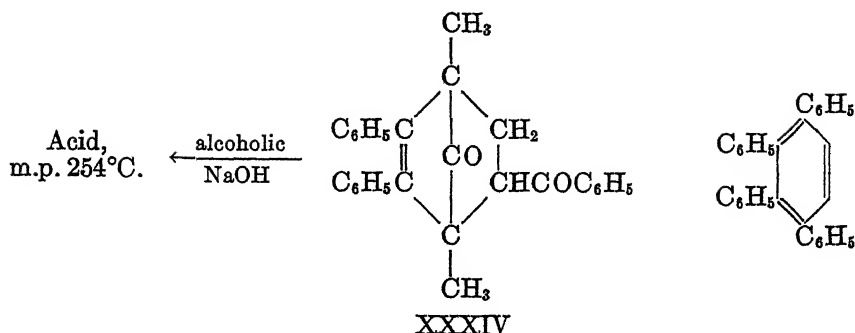
⁵ Beilstein, *Handbuch der organischen Chemie*, 4th Edition, Vol. VII: in footnote 2, page 836, it is recorded that the substance $C_{21}H_{16}O$ "could be identical with β -phenylchalcone."

The bicyclic ketones XXII and XXV are the simplest of these carbonyl bridge compounds. There is a considerable variety of bicyclic polyfunctional substances prepared by the same general reaction. The substitution of β -nitrostyrene for styrene gives a nitro derivative (XXIX); this loses nitrous acid during decarbonylation to give the aromatic hydrocarbon. Like other aliphatic nitro compounds, it can be brominated in the form of its sodium derivative. The bromonitro derivative (XXX) decomposes violently when heated; the nature of the reaction has not been determined (19).



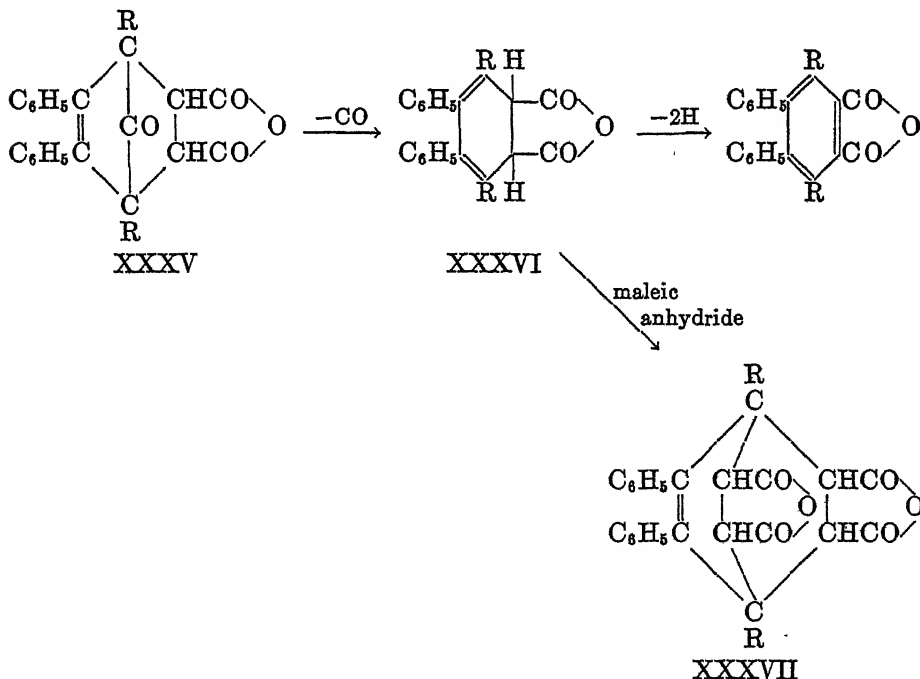
Allyl alcohol and allyl chloride have been added to tetracyclone, yielding the alcohol XXXI and chloride XXXII, respectively (1), while phenyl vinyl ketone gives the ketone XXXIII (5); the dimethyl analog XXXIV is also known





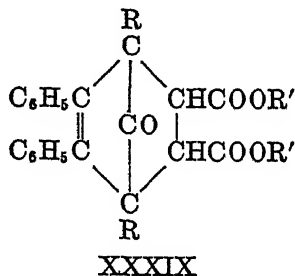
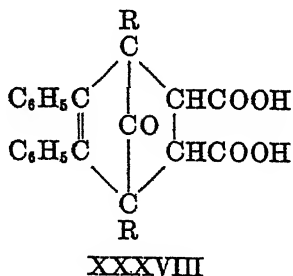
(19). Both ketones have been degraded stepwise to the corresponding benzophenones, which, in turn, were cleaved by means of sodium amide to the known hydrocarbons. The ketone XXXIV gives among other products an acid of undetermined structure upon treatment with alcoholic alkali (23) (*cf.* page 219).

The use of unsaturated anhydrides, acids, and esters gives rise to a considerable variety of polyfunctional carbonyl bridge compounds. The chemical behavior of these depends, in part, upon the substituent groups, as will be seen by the description of several individual instances. Tetracyclone and maleic anhydride readily give the bridged compound XXXV ($R = \text{C}_6\text{H}_5$) (15, 50, 51), which easily loses carbon monoxide to form the dihydrobenzene XXXVI. The latter loses the two hydrogen atoms to form the aromatic anhydride very easily; as

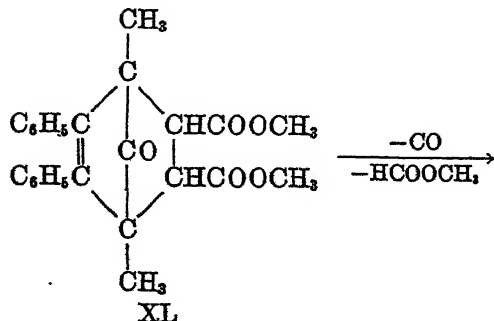


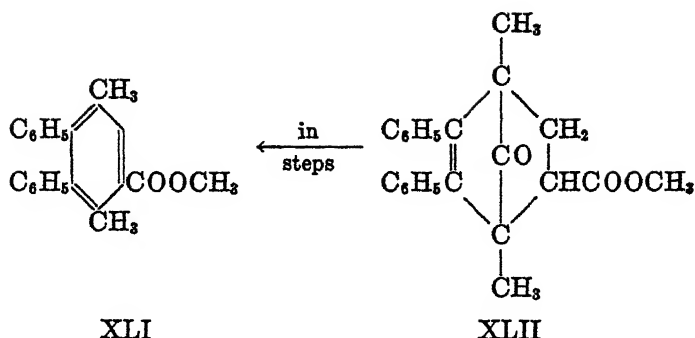
is often true in this work, if proper precautions are not observed, the intermediate products cannot always be isolated before they react to form substances other than those desired. The anhydrides can usually be converted into acids and esters.

The two conspicuous properties of the bridged anhydride are that (a) in the Grignard machine it shows one active hydrogen and two additions (15), and (b) if it is heated with maleic anhydride above the temperature of decarbonylation, a dianhydride (XXXVII) is obtained—its formation results from the addition of maleic anhydride to the dihydrobenzene derivative. The dianhydride is often secured during the initial addition reaction of maleic anhydride if suitable precautions are not observed. In order to get the relatively simple anhydride (XXXV; R = H), anhydroacetonebenzil is dehydrated by maleic anhydride at the boiling point of ethylene bromide (15), but the monomethyl homolog can be obtained by the general procedure (22). To secure the optimum yields, a trace of mineral acid is essential. The acids (XXXVIII) are usually secured from the anhydrides but in some instances can be formed directly from the components. The esters (XXXIX) are usually prepared by the addition of maleic, fumaric, or other unsaturated esters to the cyclopentadienones. The use of maleic and fumaric esters often gives rise to stereoisomers, which can be converted into the same aromatic ester. Like the previous bridge compounds, these lose carbon monoxide to give dihydrobenzene derivatives. An unusual



reaction was encountered with the *cis*-form of the ester XL, which lost methyl



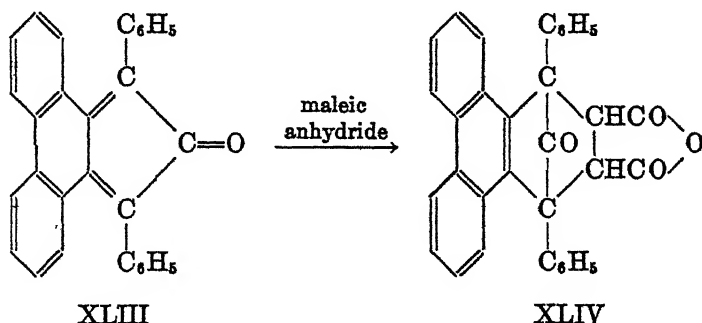


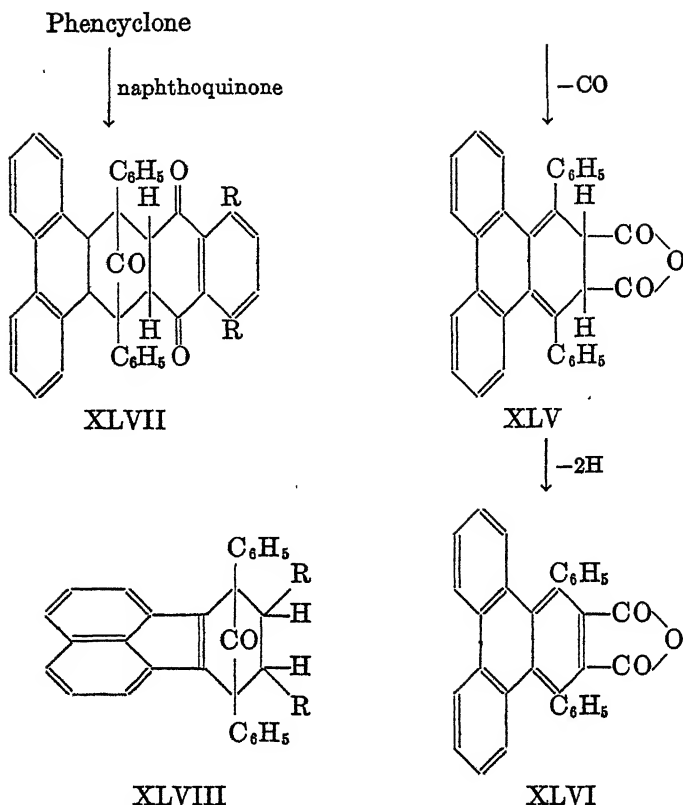
formate during decarbonylation, to give the aromatic monoester XLI (also prepared (19) by the addition of methyl acrylate to the corresponding cyclopentadienone, followed by decarbonylation and dehydrogenation of the addition product XLII). The presence of the carbonyl group in the acids, anhydrides, and esters is inferred from their analogous behavior with other carbonyl bridge compounds. An oxime has been prepared in one instance (XXXVIII; R = H) (14).

The bicyclic carbonyl bridge compounds are summarized in table 1.

(ii) *Multicyclic carbonyl bridge compounds*: For convenience in consideration, the multicyclic series of compounds can be divided into (a) the polynuclear series and (b) the indenenes and related substances.

(a) The polynuclear series: The members of this group resemble in most respects the various compounds already described. Thus, they are prepared from polynuclear cyclopentadienones, such as phenyclone (XLIII), by similar procedures. In most instances, the bridged compound has been isolated, decarbonylated, and dehydrogenated to an aromatic structure. In illustration, maleic anhydride in benzene or chlorobenzene adds to phenyclone; the addition product (XLIV) loses carbon monoxide and hydrogen, so that the dihydroanhydride (XLV) is never obtained completely pure. The aromatic derivative (XLVI), however, is easily prepared (41).





Quinones have been added in similar fashion (XLVII) (24, 45); decarbonylation was carried out in boiling nitrobenzene, probably because it resulted in a cleaner product.⁶ Acecyclone gives carbonyl bridge compounds (XLVIII) less frequently, for the temperature of decarbonylation is often below that at which they are formed; consequently, the polynuclear dihydro and aromatic fluoranthene derivatives are obtained directly (5, 39).

The multicyclic polynuclear carbonyl bridge compounds are summarized in table 2.

(b) The indenenes and related substances: Historically, these comprise the earliest known carbonyl bridge compounds, and were the first to have their structures elucidated.

Cyclopentadiene adds to tetracyclone; although the addition product (XLIX) regenerates the components when warmed, it gives a dibromide, and is reducible to a cyclopentane derivative (L). The latter can no longer reverse on warming, so that it loses carbon monoxide in boiling cymene solution (56). The presence of the six-membered carbon ring was shown by degradation after aromatization

⁶ Since nitrobenzene serves as a dehydrogenating agent, it is frequently used in diene syntheses which cannot otherwise be realized. The aromatization of the dihydro addition products removes them from the equilibrium and the reaction runs to completion.

TABLE 1

Unsaturated bicyclic compounds having a carbonyl bridge

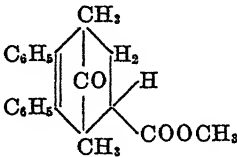
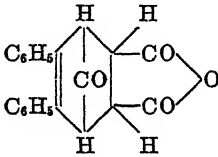
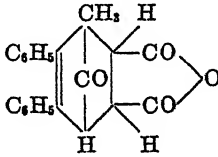
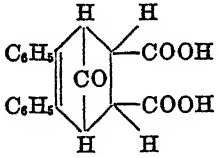
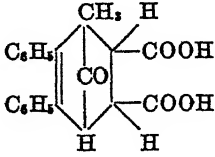
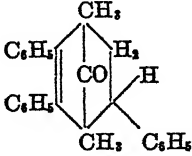
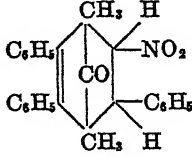
NO.	SUBSTANCE	REFERENCE
XLII		(19)
XXXV; R = H		(15)
		(22)
		(15)
		(22)
XXV		(19)
XXIX		(19)

TABLE 1—*Continued*

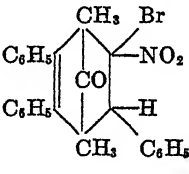
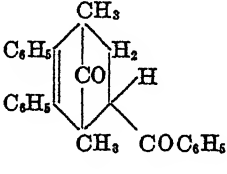
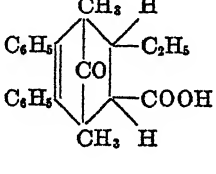
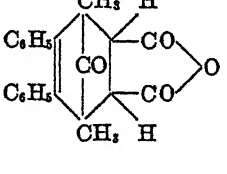
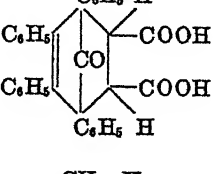
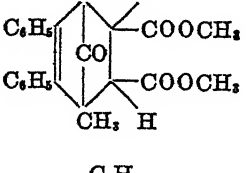
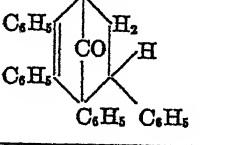
NO.	SUBSTANCE	REFERENCE
XXX		(19)
XXXIV		(19)
XXXV; $\frac{5}{2}R = CH_3$		(19)
		(19)
		(15)
XL		(19)
XXII		(20)

TABLE 1—*Continued*

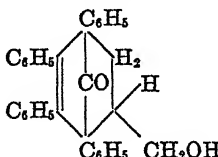
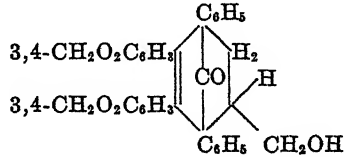
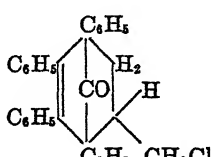
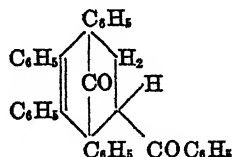
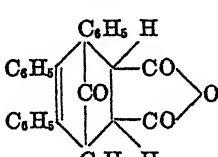
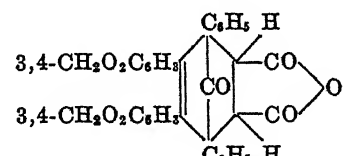
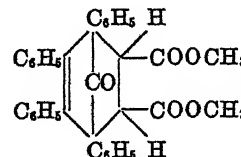
NO.	SUBSTANCE	REFERENCE
XXXI		(1)
		(96)
XXXII		(1)
XXXIII		(5)
XXXV; R = C ₆ H ₅		(15)
		(96)
XXXIX; R = C ₆ H ₅ R' = CH ₃		(15)

TABLE 2
Multicyclic compounds (polynuclear series)

NO.	SUBSTANCE	REFERENCE
		(97)
		(97)
		(97)
		(5)
		(5)
		(1a)

TABLE 2—Continued

NO.	SUBSTANCE	REFERENCE
XLIV		(1a)
		(41)
LXXXV		(40)
		(97)
		(97)

TABLE 2—Continued

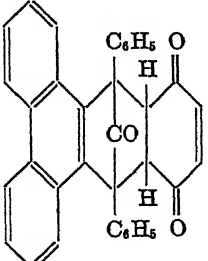
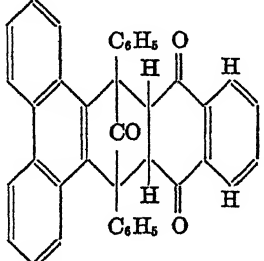
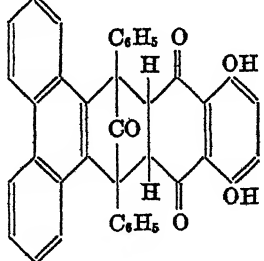
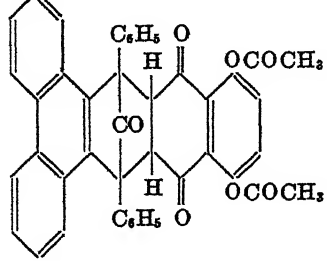
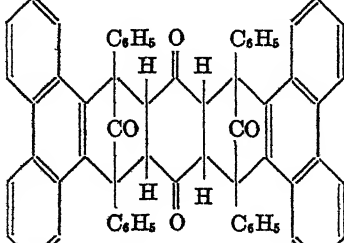
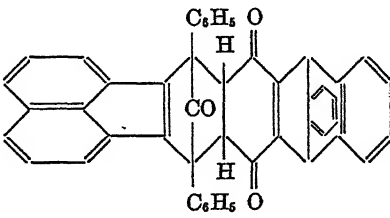
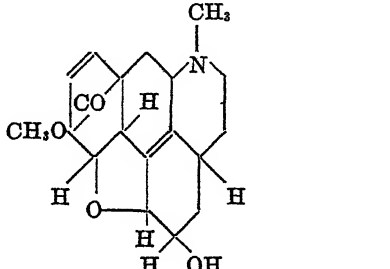
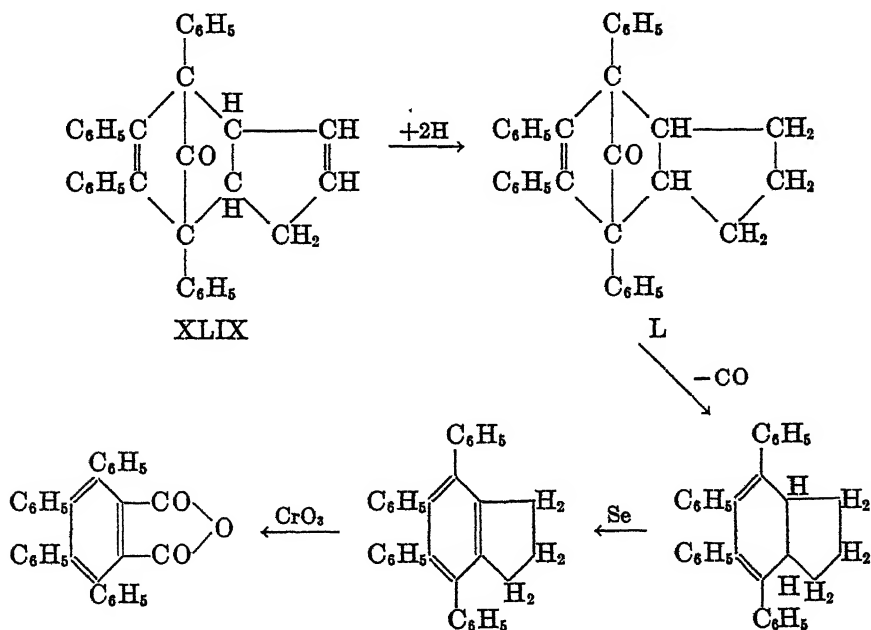
NO.	SUBSTANCE	REFERENCE
		(24, 25)
XLVII; R = H		(24, 45)
XLVII; R = OH		(45)
XLVII; R = OCOCH ₃		(45)
		(24)

TABLE 2—Concluded

NO.	SUBSTANCE	REFERENCE
LX		(1a)
		(71)

in the usual way. The presence of the carbonyl group in both XLIX and L was detected in the Grignard machine; each substance consumed one equivalent of reagent without evolution of gas, i.e., underwent one addition (23).



As was mentioned earlier, Japp's anhydroacetonebenzil (XVI) gave a bi-molecular product with acidic dehydrating agents, according to the equation:

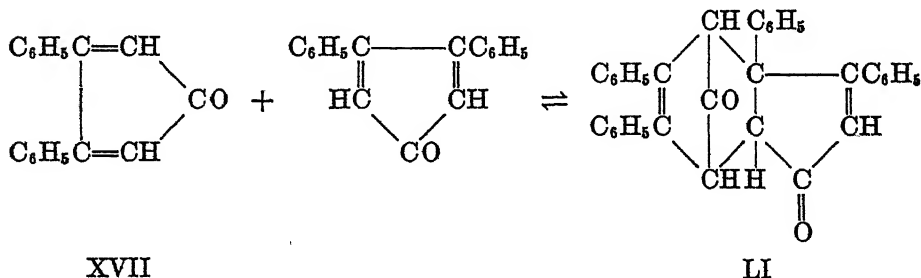


The doubled formula was originally adopted because the substance, when heated, evolved one equivalent of carbon monoxide and gave a product that formed a phenylhydrazone (60), thus:



Subsequent cryoscopic molecular-weight determinations in benzene gave values of 463 and 473, whereas the calculated value is 464 (62). The dimeric substance did not give a derivative with phenylhydrazine. Homologous bi-molecular products were later obtained with α - and β -methylanhydroacetonebenzils (63), α,β -dimethylanhydroacetonebenzils (54), and amylanhydroacetonebenzil (22). It is important to have a trace of mineral acid present to effect dehydration, for *pure* anhydroacetonebenzil can be distilled unchanged (84). No structures were suggested for the bimolecular product.

In 1933, Allen and Spanagel proposed a structure for the bimolecular product, as a result of their investigation of anhydroacetonebenzil and related compounds. Noting that diphenylcyclopentadienone (XVII), the dehydration product of anhydroacetonebenzil, was both a diene and a substance containing an ethylenic linkage, they suggested that a diene synthesis occurred (17, 18). The new structure (LI) contains a six-membered carbon ring having a carbonyl bridge and an angular phenyl group, and can be considered to be an indenone. The

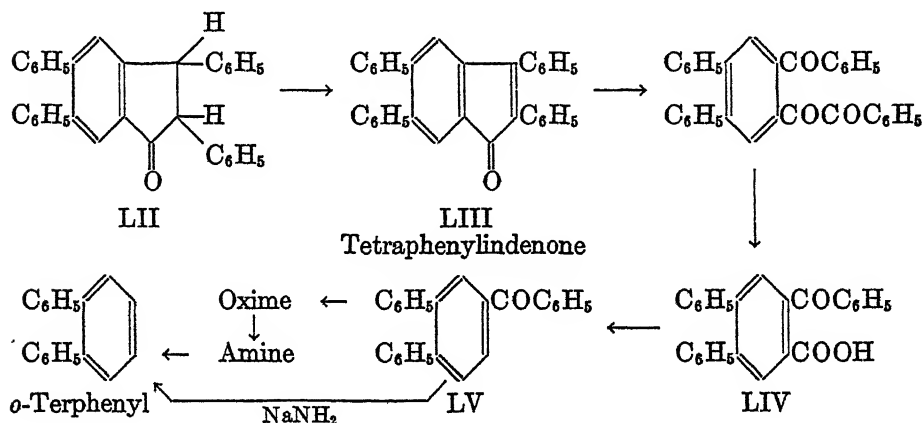


essential correctness of this structure was shown through a long degradation that terminated in the known *o*-terphenyl; this degradation will be given in detail, since it illustrates the difficulties inherent in dealing with such multi-cyclic systems.

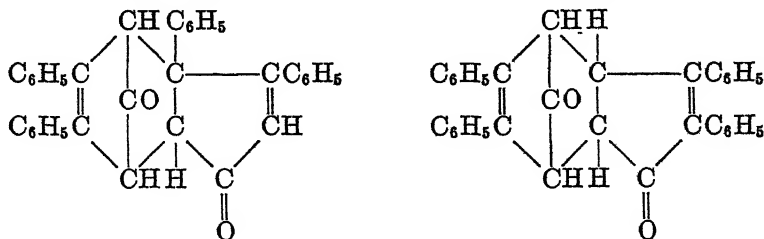
In the first place, the structure LI represents the molecule as having two carbonyl groups, which are linked differently and hence would be unlike in behavior. The bimolecular product loses carbon monoxide, a reaction which indicates dissimilar linkings, and it is a diketone for it forms a dioxime, which exists in stereoisomeric forms. As Japp found, the decarbonylated product is a ketone, for it forms a phenylhydrazone. Allen and Spanagel made the assumption that this ketone was the indanone LII, and this was later found to be correct. To account for its formation from the bimolecular product, it was proposed that

there had been a 1,3-rearrangement of a phenyl group. Although the reaction was unrecognized at that time, other instances have since been discovered (11, 20, 21).

The degradation of the indanone proceeded as follows (18): It was first dehydrogenated by the use of sulfur to the red tetraphenylindenone (LIII), and the latter was then oxidized to a triketone in which two carbonyl groups are adjacent. This was cleaved by alkaline hydrogen peroxide to the keto acid LIV, which was then decarboxylated. The oxime of the resulting ketone (LV) was submitted to a Beckmann rearrangement, and the aminoterphenyl produced deaminated through the diazo reaction to *o*-terphenyl; the latter was identical with an authentic specimen. Later on, the ketone was cleaved directly to *o*-



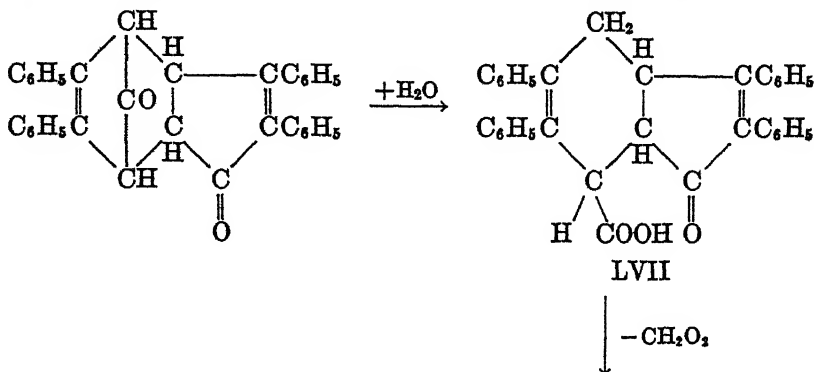
terphenyl by means of sodium amide (5), thus avoiding the troublesome manipulations previously encountered. Subsequently, all these degradation products were synthesized by independent reactions (5, 10). The formation of *o*-terphenyl proves the presence of a six-membered ring with two phenyl groups in the ortho-position to each other. The location of the side chain is clear from the keto acid LIV, and the other two phenyl groups are located in the 2- and 3-positions in the indenone LIII. It must be concluded, therefore, that during either the formation of the bimolecular product or the decarbonylation, there has been a 1,3-shift of the phenyl group; the first alternative appears to be more probable. The structure for the bimolecular product shown in LI was accepted by others interested in the field (30, 38), but the stage at which the phenyl shift occurred was not determined. Allen and Gates (8) continued the experimental work and



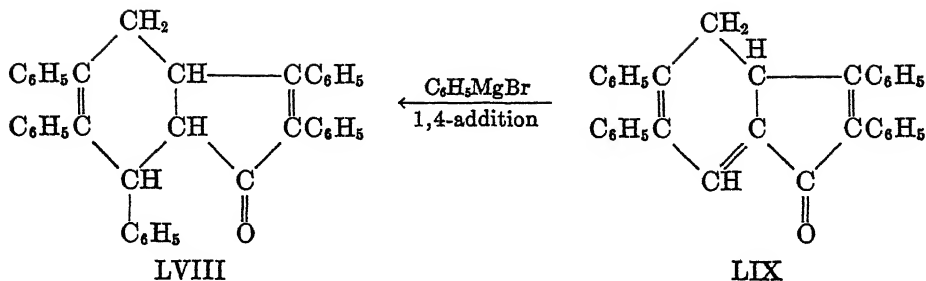
from the results concluded that the correct structure was that shown in formula LVI.

In the first place, the bimolecular product has the expected molecular weight (62). Second, the phenyl groups are, beyond any reasonable doubt, in the 2- and 3-positions in the indenones. Third,⁷ although the bimolecular product was formed at a relatively low temperature, an independent check was considered desirable—this was found in its behavior with alkaline reagents (9), a type of reaction already described (page 219). In the present instance, it is of special interest to note that although there are two carbonyl groups in the bimolecular product, the reaction takes place only with the carbonyl bridge.

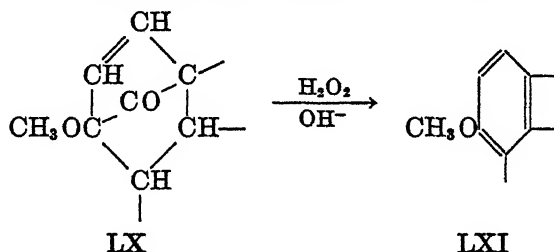
The bimolecular product, after treatment with methyl alcoholic potassium hydroxide, gives an acid (sodium alkoxides give the corresponding esters), the analysis of which shows the addition of a molecule of water. Since the acid can no longer be decarbonylated, it is the carbonyl bridge that has become converted to a carboxyl group. Oxidation of the acid (as the sodium salt) results in the loss of CH_2O_2 (formic acid?) and formation of a dienone (LIX). The dienone is an α,β -unsaturated ketone, for it gives a ketone (LVIII) when treated with the Grignard reagent; the formation of a ketone indicates the presence of an ethylenic linkage in the position alpha to the carbonyl group. From these facts it is inferred that the acid is correctly represented by structure LVII. (Although there is, to be sure, another α,β -unsaturated system involving the indene ring, the possibility of 1,4-addition to this is excluded because of the observation that other indenones containing the same system give only carbinols, formed by 1,2-addition.) The oxidation results are accounted for by a retrograde Michael reaction, in which formic acid is split off and destroyed by the oxidizing agent. An additional instance of this reaction is given on page 240.



⁷ At the time the structure LI was proposed, one of the arguments that appeared to be in its favor was the formation of thiophenol when the substance was heated with sulfur; by analogy with Ruzicka's terpene work (72), the assumption was made that sulfur would remove the angular group as thiophenol. This argument lost its force when it was found that thiophenol was obtained in other sulfur dehydrogenation reactions where there was no reasonable doubt as to the absence of an angular phenyl group (8).



This sort of cleavage has an analogy in morphine chemistry; Rakshit (71) found that porphyroxin (LX), upon treatment with sodium hydroxide and hydrogen peroxide, lost CH_2O_2 and gave codeine (LXI) (only partial formulas are

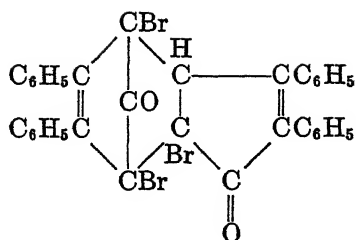


used for convenience). Porphyroxin is an unsaturated carbonyl bridge compound of exactly the same type as those under discussion; unfortunately, the author did not examine its behavior on heating. It does give the usual carbonyl derivatives (oxime, phenylhydrazone, semicarbazone). It is of particular interest to note that the double bond of porphyroxin methyl ether can be reduced, and that the saturated ketone can no longer be cleaved by the alkaline oxidizing agent.⁸

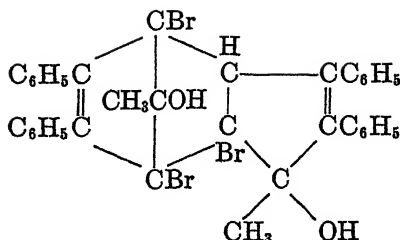
The bimolecular product, then, is represented by the structure LVI. Any open-chain structures for dimers of the diphenylcyclopentadienones are excluded by most of the reactions that have been described, as well as by the fact that α,β -dimethylanhydroacetonebenzil, which forms a dimer, could not give rise to any of the possible open-chain structures. It does not seem likely that the bimolecular product is a mixture, even though it forms two dioximes, because it is regenerated from both of these with the same melting point as the original dimer.

Owing to its many functional groups, the bimolecular product undergoes other reactions. There are three hydrogen atoms in the position alpha to carbonyl groups, all of which can be replaced by bromine (8). The tribromoketone formed gives a dicarbinol with methylmagnesium iodide, showing that it still has two carbonyl groups. When treated quantitatively with methylmagnesium iodide in the Grignard machine, the bimolecular product gives anomalous results, one active hydrogen and one addition (18); the methane is evolved only

⁸ Some doubt has been expressed recently as to the existence of porphyroxin as a definite chemical entity (93).

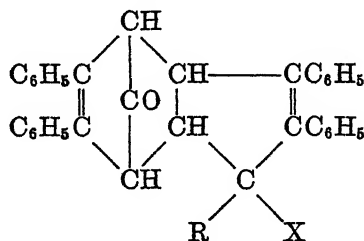


LXII



LXIII

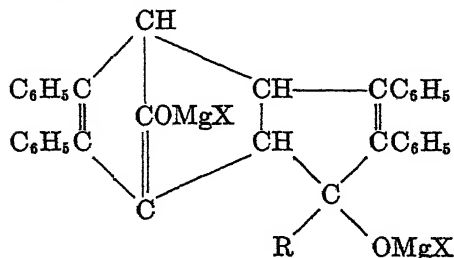
during the heating period, a reaction which indicates that enolization is taking place but that the enol is not already existent. The bimolecular product is not regenerated upon acidification; instead there is formed a carbinol (LXIV), which



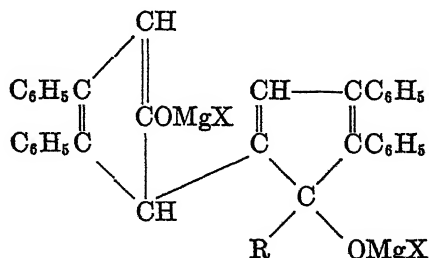
LXIV

still has the carbonyl bridge, for it can be decarbonylated and dehydrated to a hydrocarbon of known structure (when $R = C_6H_5$ and $X = OH$, a polyphenylindene results) (11). This indicates that addition has taken place with the indenone carbonyl group. Whether the complex is a real enolate or not, the carbonyl group is obviously covered in some way, for any exposed group would never survive the huge excess of Grignard reagent used.

A bimolecular product which has no available hydrogen does not give off gas with methylmagnesium iodide (19). Thus, it appears that the carbonyl bridge and one of the hydrogen atoms in the position alpha to it are involved. The structure of the magnesium complex is uncertain; as first proposed (8) (LXV), all the facts are accounted for, but the presence of a double bond at the bridgehead is at variance with Bredt's rule (27).⁹



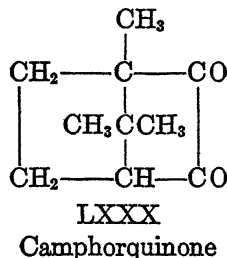
LXV



LXVa

⁹ Carvopinone is generally believed to have a double bond at the bridgehead (94).

An alternate possibility is shown in formula LXVa, in which it must be assumed that one ring has been opened under the influence of the Grignard reagent. Upon acidification, recyclization must take place, for the product can be decarbonylated easily. Evidence in its favor is the behavior of camphor-



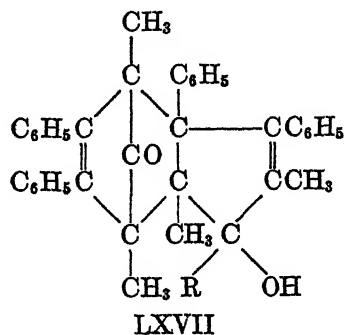
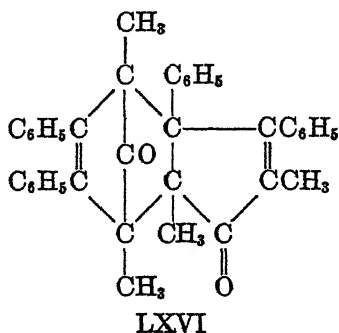
quinone (LXXX). This substance has a carbonyl group and adjacent alpha hydrogen, and if its behavior paralleled that of known hindered 1,2-diketones (25a, 25b), it would be expected to be practically completely enolized. Its enol would have to have a double bond at a bridgehead. With methylmagnesium iodide, camphorquinone evolves no gas but shows two additions (23); this indicates that enolization is not likely to occur, if it involves the formation of a double bond at a bridgehead. This analogy was suggested by Woodward (85a), who also favors a structure of the type of LXVa for the magnesium enolate.

The open-chain structure for the bimolecular product, corresponding to the enolate LXVa and analogous to that of dipyrroles (11a), was considered previously and discarded because (a) no such structure is possible in the case of the dimer obtainable from α,β -dimethylanhydroacetonebenzil, (b) the bimolecular product does not react with aromatic aldehydes, and (c) the easy decarbonylation can be accounted for only by assuming a prior cyclization.

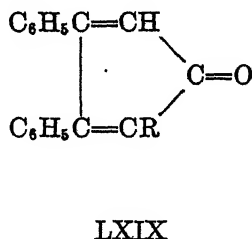
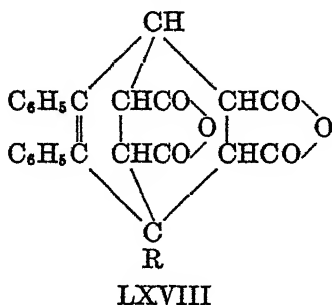
Other bimolecular products, formed by dimerization of cyclopentadienones, are known, but they must have structures corresponding to LI, because they all dissociate to varying degrees in solution or when heated; hence, there have been no rearrangements. For example, Dilthey (44) observed that 2,3,5-triphenylcyclopentadienone was colorless in the solid state, but turned red at the melting point; the values (601, 575, 439, 394, 349) found for the molecular weight were between those for the dimeric (616) and the monomeric (308) formulas. He concluded that this was an intermediate case between the colorless dimeric 3,4-diphenylcyclopentadienone (the bimolecular product described) and the deep red monomeric tetraphenylcyclopentadienone; in considering structures, the possibility of the occurrence of a diene synthesis was mentioned.

Allen and VanAllan (19) found that the dimeric 2,5-dimethyl-3,4-diphenylcyclopentadienone (LXVI) gave products the nature of which indicated that it had partially dissociated to the monomeric form. It gave abnormal values for molecular weight (table 3, page 245), which were taken to indicate about 20 per cent dissociation. The observation that the boiling benzene solution was highly colored, although the solid was white, was interpreted as a confirmation of the assumption that dissociation had occurred, for the known monomeric

tetraphenylcyclopentadienone is deep red. The dimethylated dimer forms a hexabromide, the structure of which is unknown, with evolution of hydrogen bromide, and it gives indenols (LXVII) which still have the carbonyl bridge, for they can be decarbonylated; thus, it does not dissociate in all its reactions.



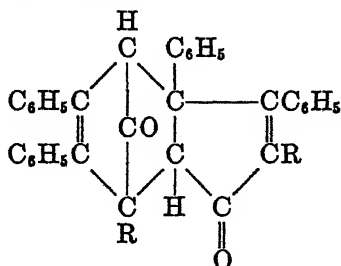
Bimolecular products are obtained from monomethyl- and *n*-amyl-anhydroacetonebenzils (22). They appear to be intermediate in properties between the unassociated tetracyclone and the dimerized dimethyl analog (LXVI). The benzene solutions are colorless and there is no addition of maleic anhydride in this solvent, but in boiling trichlorobenzene some carbon monoxide is evolved and a mixture of two anhydrides is obtained. One of these is a dianhydride (LXVIII), which corresponds to the one obtained from the other anhydroacetonebenzils, and which can only have been obtained by a dissociation of the bimolecular product into the monomer LXIX, prior to the addition. The other is a monoanhydride derived from the decarbonylated indenone which has lost both carbon monoxide and a molecule of benzene.



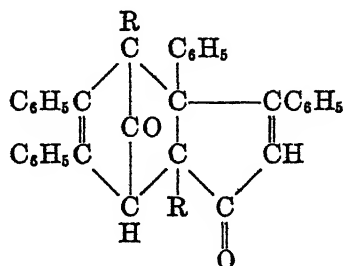
Since all these substituted dimers dissociate to give products identical with those derived from the corresponding monomeric anhydroacetonebenzil, it is clear that there has been no rearrangement of a phenyl group; the original bimolecular product, therefore, is an exception. Thus, the difference in the dissociation of the dimers derived from unsubstituted, monosubstituted, and disubstituted anhydroacetonebenzils is one of degree only.

Without regard to the possible existence of stereoisomers, the dialkylated bi-

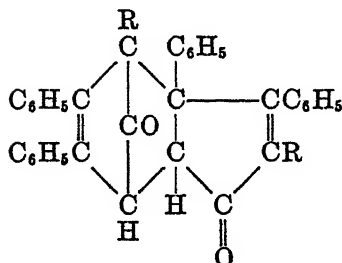
molecular products obtained from the monoalkylated anhydroacetonebenzils can have four structures, LXX-LXXIII, depending upon which side of the addend molecule LXIX is used. In order to decide upon one structure among them it is necessary to compare the chemical properties with those of the analogous bimolecular products.



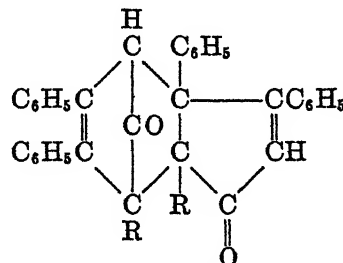
LXX



LXXII



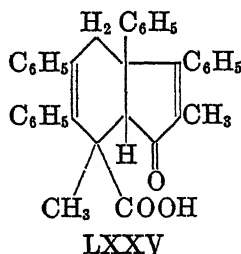
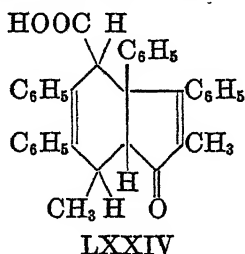
LXXI



LXXIII

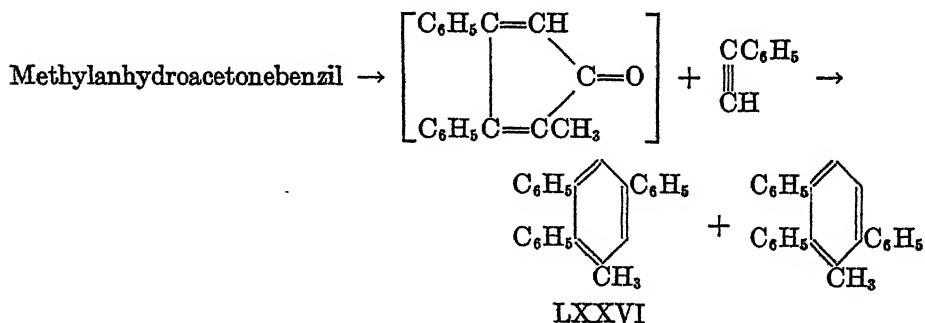
The fact that the bimolecular product dissociates so that a derivative of the monomolecular dienone can be obtained indicates that there has been no rearrangement; that is, it resembles the bimolecular product (LXVI) of dimethyl-anhydroacetonebenzil. The bimolecular product (LVI) from anhydroacetonebenzil, however, has been shown to differ from the anticipated structure (LI) in the location of a phenyl group, which migrated from the angular position to the 2-position, as discussed previously. Such a rearrangement is possible because of the presence of the hydrogen atom at this point on the indenone ring—with which the phenyl group appears to interchange. The available evidence on angular phenyl groups indicates that they show a great tendency to migrate when this is possible, but there is, as yet, no single instance of a shift involving a displacement of any atom or group other than hydrogen. It may, therefore, be concluded that if there is no rearrangement, there is no hydrogen atom in the 2-position of the indenone ring. Hence, structures LXXII and LXXIII can be excluded. Since all dimerized cyclopentadienones have been formed from anhydroacetonebenzils having phenyl groups in the 2- and 3-positions, it follows that in those bimolecular products that show dissociation there must be an angular phenyl group. The proof of the presence of such a group is secured in the same degradation reactions used to distinguish between the structures LXX and LXXI.

When the dimethylated bimolecular product from α -methylanhydroacetonebenzil is treated with alcoholic sodium hydroxide, a reaction that has been shown to cleave the carbonyl bridge at one end (7), two isomeric acids are obtained (22). The formation of these two acids may be taken to indicate that cleavage has taken place on both sides of the bridge. If structure LXX is arbitrarily selected for the bimolecular product (though this might be considered to have a preference over LXI because its formation would be less sterically hindered), the two acids may be represented by LXXIV and LXXV.



It has previously been shown (7) that a γ -carboxylic ketone of the type of LXXV loses CH_2O_2 upon treatment with potassium permanganate. One of these acids exhibits this behavior, and gives a ketone; consequently, to this acid is assigned the structure LXXV.

The second acid, upon similar treatment with permanganate, loses $\text{C}_{10}\text{H}_{10}\text{O}$ and gives an aromatic acid, $\text{C}_{26}\text{H}_{20}\text{O}_2$; this reaction strongly indicates that the methyl and carboxyl groups are not attached to the same carbon atom, so the structure LXXIV is assigned to this acid. Upon decarboxylation, the aromatic acid gives a hydrocarbon, $\text{C}_{25}\text{H}_{20}$. This hydrocarbon is 2,3,5-triphenyltoluene, which can be synthesized easily from methylanhydroacetonebenzil and phenylacetylene. The synthesis is ambiguous, in that two isomeric hydrocarbons could



be formed, owing to the unsymmetrical nature of the components. However, only one product is obtained, and it is identical with the triphenyltoluene formed from the degradation of the dimer. This identity, moreover, definitely eliminates structures LXXII and LXXIII. It also proves the existence of a six-membered ring in the bimolecular product.

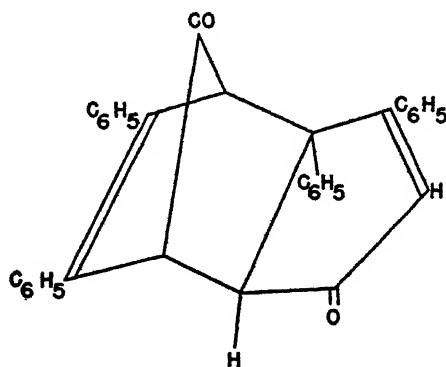
The presence of the third phenyl group, the location of which with respect

to the other two is established by the synthesis, is very significant. It furnishes unequivocal proof that there is an angular phenyl group in the acid formed by cleavage of the bridge, and thus in the bimolecular product itself. The presence of this angular phenyl group has previously been inferred from a wealth of evidence, but this is the first time it has been clearly demonstrated.

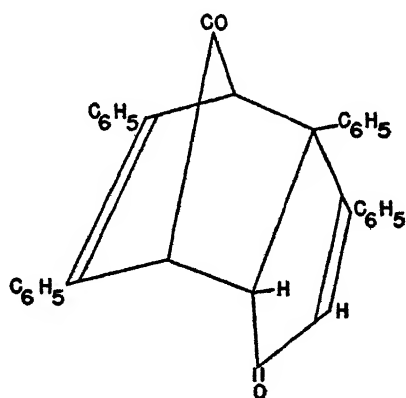
As the two acids that could be derived from LXXI by a similar cleavage of the carbonyl bridge would not be expected to give both these reactions just described (barring a transannular elimination which seems to us extremely unlikely), that structure is eliminated from consideration; the preferred structure for the dimer is that shown in LXX.

Since all the known dimerized cyclopentadienones have been formed from anhydroacetonebenzils having phenyl groups in the 2- and 3-positions, it follows that in those bimolecular products that show dissociation there must be an angular phenyl group.

From an inspection of the structural formula assigned to the bimolecular product, as it appears written in the plane of the paper, it is obvious that stereoisomeric forms are possible. When one views the three-dimensional multi-cyclic system, the position of the ring planes in space gives rise to a variety of



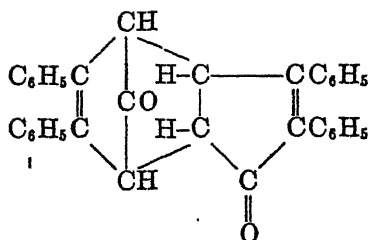
LXXVII
exo



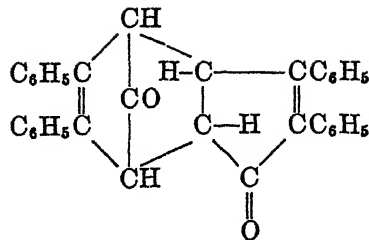
LXXVIII
endo

stereoisomers that have been called endo and exo (70d). Practically always only one form is produced in a reaction, and when its configuration has been determined, it has been endo. Such forms are shown in formulas LXXVII and LXXVIII. By analogy with the behavior of cyclopentadiene, the bimolecular products from the cyclopentadienones are assumed to be endo, with the reservation that such a generalization is not absolute. A stereoisomeric substance, which may have the exo configuration, was obtained from the endo form by the action of certain oxidizing agents.

Stereoisomeric forms are also possible in which the rings are joined in *cis* and *trans* positions. Interconversion of the two forms could be visualized as possible through a process of enolization, as Hückel was able to realize quantita-



LVia



LVib

tively in the conversion of *cis*-decalone to the *trans*-form (57).¹⁰ The bimolecular product obtained from anhydroacetonebenzil was assigned the *cis* structure (LVia) on the inconclusive evidence that the product formed on decarbonylation readily added maleic anhydride; such an addition is in accordance with the *cis* principle. From an examination of models it was observed that this particular bicyclic ring system linked in the *trans* positions could not add maleic anhydride without a distortion which is so great that it practically rules out such a possibility.

When the bimolecular product is stirred at room temperature with cold alkaline hydrogen peroxide, it gives a "peroxide" containing four extra atoms of oxygen; while the product seems stable at room temperature, it decomposes violently when heated. This new peroxide liberates iodine from potassium iodide in acetic acid, and bromine from hydrobromic acid, the starting material being regenerated. However, when it is dissolved in acetic acid, oxygen is liberated, and an isomer of the starting material results (9).

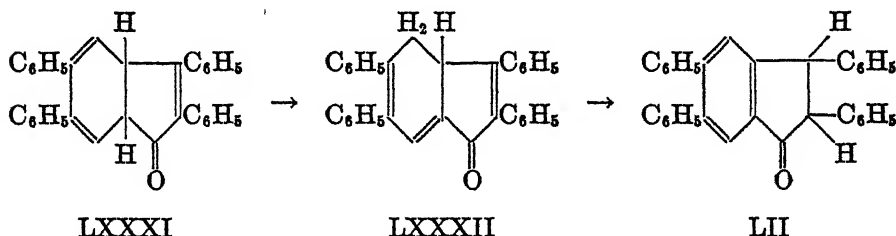
This isomer still contains a carbonyl bridge, for it evolves carbon monoxide when heated; the other product is an indanone (LXXIX) isomeric with LII (13). All other reactions are the same as that of the starting material: e.g., it gives the same acid with alcoholic potassium hydroxide, shows one active hydrogen and one addition, and gives the same carbinol with phenylmagnesium bromide. That is, reactions used to prove structure indicate that the groups are linked alike in both substances; the difference between the two, therefore, must be of a spatial nature. The fact that both give the same derivatives can be accounted for by interconversion catalyzed by acidic or basic substances present in all reactions. The only reaction carried out in the absence of catalysts is the pyrolysis; in each case, this leads quantitatively to a different substance.

When the original bimolecular product (LVI) was treated with chromium trioxide in acetic acid, varying but poor yields of oxygen-containing substances were obtained (13); the latter were not of constant composition, never containing as much as four atoms of oxygen, but all showed the same behavior in reactions, as described in the preceding paragraph. It appears that oxidation of this type

¹⁰ This easy interconversion was explained as proceeding through the enol (which would have a double bond at a bridgehead and not be in accord with Bredt's rule). Whether a parallel reaction would take place in the hydrindone series is unknown, since only the β -ketone has been examined (58).

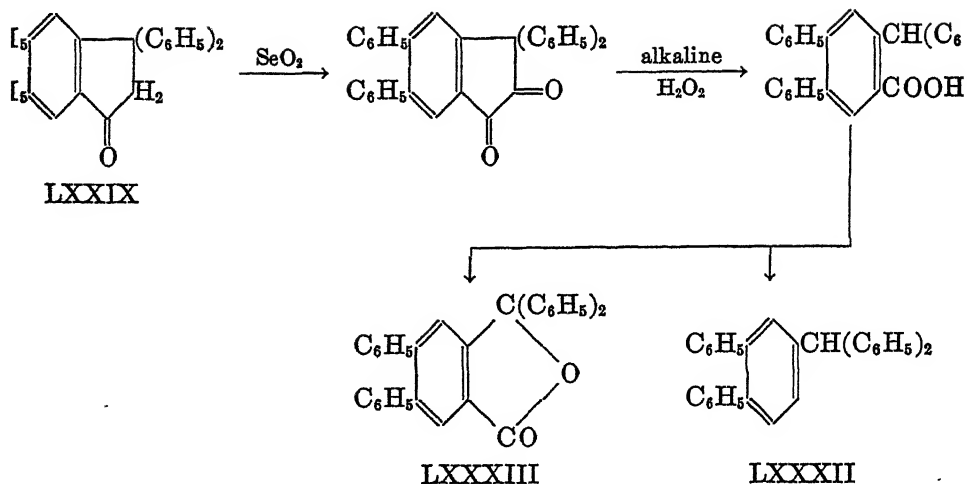
of carbonyl bridge compound yields some sort of peroxide, and is thus unsuitable for proving structure in the ordinary way. It will be recalled that the results of the Grignard machine examination indicated one enolizable hydrogen, and that enolic forms (LXV and LXVa) were suggested. Now, since Kohler has shown (65) that enols of unsaturated ketones easily form peroxides, the instances just cited find some support. These two anomalous properties, enolization and peroxide formation, must be connected in some way with the presence of the carbonyl bridge and an adjacent hydrogen atom, as well as space relations, for the new isomer which is formed from decomposition of the peroxide does not itself form a peroxide. At the present time, the structure of these oxygen-containing substances is left in abeyance, but, presumably, one oxygen atom has added to each ethylenic linkage to give a multicyclic polyoxido system. Whether the bimolecular product and its isomer are of the *exo,endo* or *cis,trans* types is unsettled.

The products of decarbonylation of both isomers must next be described. Although, as outlined, Japp (60) obtained the dihydroindanone LII directly, Allen and Spanagel (18) were able to isolate an isomeric indenone which was easily rearranged to Japp's substance. Furthermore, Allen and Gates (7) obtained an additional indenone (LXXXII) and showed that it was an intermediate between the two, LXXXI and LII. The only difference in the three isomers is in the location of double bonds and hydrogen atoms. The first dienone was assigned the structure LXXXI, for it added maleic anhydride; the evidence for the others has already been given. The sequence terminating in Japp's indanone is accomplished in the laboratory by heat and/or acidic catalysts. Probably it goes in the direction indicated, because each form has an increased



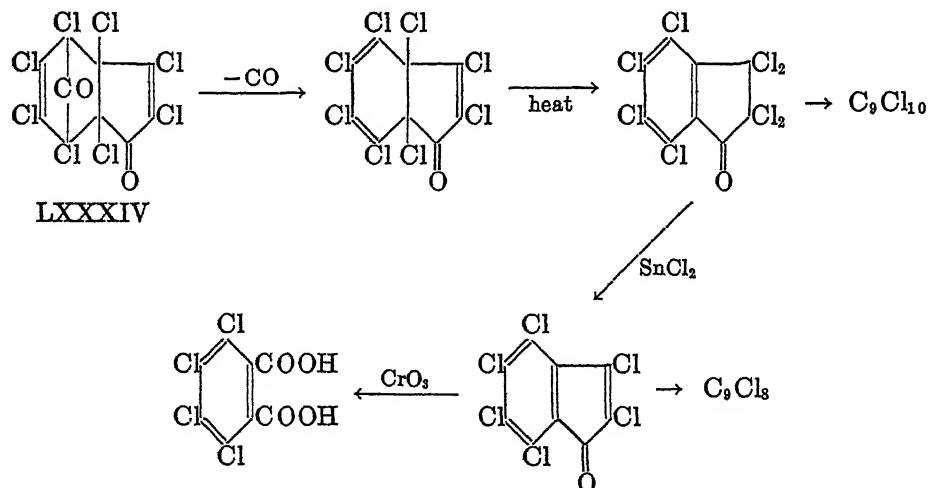
stability; the isolated double bonds (one pair conjugated) first become a long conjugated system (LXXXI) and then aromatize.

The isomeric indanone formed on decarbonylation of the *trans*-isomer has the structure LXXIX. This was shown by degradation to the known substances LXXXII and LXXXIII, which were synthesized independently (9). Analogous reactions of indanones lacking the *Ar*-phenyl groups were exactly parallel. In the structure thus established, there are now two phenyl groups on one carbon atom. It must be concluded, therefore, that in this instance there has been a 1,2-shift of a phenyl group;



apparently, configuration can be a determining factor in the nature of rearrangements.

A completely chlorinated carbonyl bridge compound in the indenone series, LXXXIV, was discovered by Zincke many years ago (89). Its structure was established by a stepwise degradation, eventually producing tetrachlorophthalic acid. The carbonyl group in the indane-indenes was detected by means of phosphorus pentachloride (88, 90). The similarity between this series and the



bimolecular product LVI should be noted, in particular that here there is a 1,3-shift of chlorine, comparable to the 1,3-phenyl rearrangement.

An as yet unexplained feature is the observation that molecular-weight determinations of the bimolecular products, while satisfactory in benzene and other solvents, are unreliable in boiling carbon tetrachloride. The values are given in table 3.

Another noteworthy feature encountered in the work with carbonyl bridge compounds is the frequency with which they retain solvent of crystallization. The most conspicuous examples of solvents are benzene and acetic acid. Prolonged drying *in vacuo* is required to obtain good analytical figures from substances that exhibit this behavior.

(c) Other types of addends

(i) *Acetylenic compounds*: Earlier in this article it was pointed out that substances containing an acetylenic linkage would add to cyclopentadienones. With this class of addends, the carbonyl bridge is usually eliminated during the reaction, and the substance formed is a dihydrobenzene that is easily dehydrogenated to give an aromatic structure. In this way a large number of polyarylated benzene derivatives has been secured (15, 19, 22, 37, 40, 42, 43, 49, 50, 51).

There is no doubt that a carbonyl bridge compound is an intermediate, but

TABLE 3
Molecular-weight determinations of bimolecular products

	MELTING POINT	MOLECULAR WEIGHT		
		Calculated	Found	
			In C_6H_6	In CCl_4
Unsubstituted; LI.....	206	464	463* 463, 473†	388, 385
Dimethyl.....	230	492	461*	400
Di- <i>n</i> -amyl	154	604	578*†	491
Tetramethyl; LXVI (19).....	181	520	414, 417*	470, 380

* Ebullioscopic (22).

† Cryoscopic (62).

‡ In ethanol*, 635; in chloroform*, 594, 587; in methylene chloride*, 590 (22).

the temperature of reaction is above that of decarbonylation. An explanation of the easy cleavage will be discussed later. In one instance, the carbonyl bridge compound was isolated—when phenylpropionic acid added to phencyclone (LXXXV) (40) (see page 257).

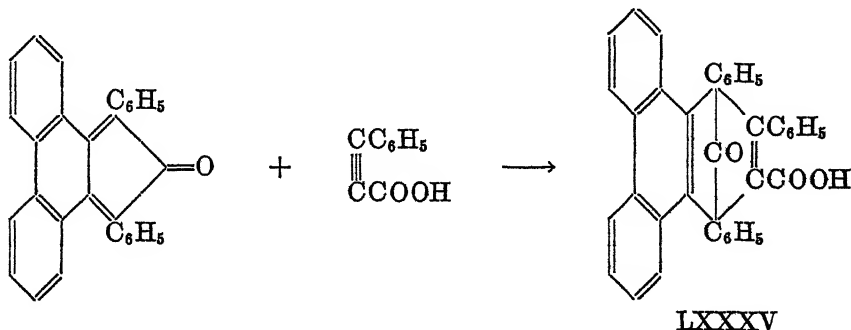


TABLE 4
Multicyclic compounds (indene series)

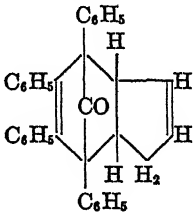
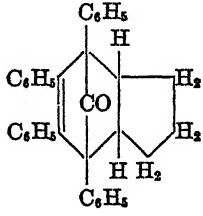
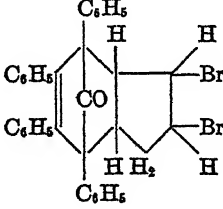
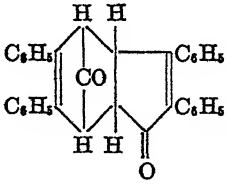
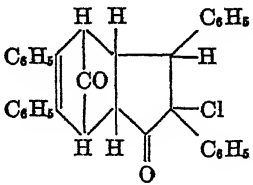
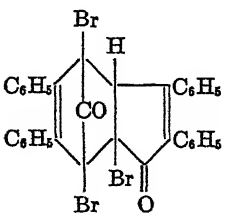
NO.	SUBSTANCE	REFERENCE
XLIX		(56)
L		(56)
		(56)
LVI		(7, 13, 18, 60)
		(13, 30)
LXII		(8)

TABLE 4—Continued

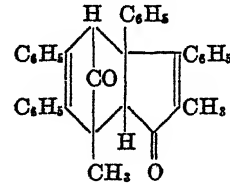
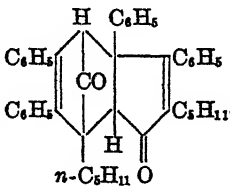
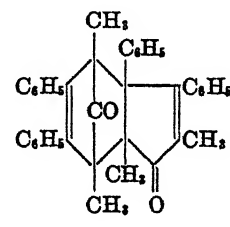
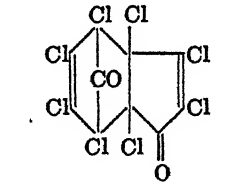
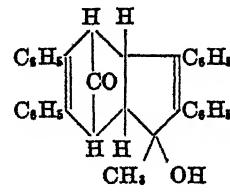
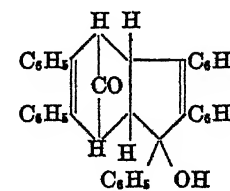
NO.	SUBSTANCE	REFERENCE
LXX; R = CH ₃		(63)
LXXI; R = <i>n</i> -C ₆ H ₁₁		(22)
LXVI		(54)
LXXXIV		(88)
LXV; R = CH ₃ X = OH		(8)
LXV; R = C ₆ H ₅ X = OH		(8)

TABLE 4—Continued

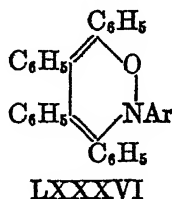
NO.	SUBSTANCE	REFERENCE
LXV; R = α -C ₁₀ H ₇ X = OH		(8)
LXVII; R = CH ₃		(19)
LXVII; R = C ₆ H ₅		(19)
LXV; R = CH ₃ X = Cl		(8)
LXV; R = α -C ₁₀ H ₇ X = Br		(8)
LXV; R = C ₆ H ₅ X = OCOCH ₃		(8)

TABLE 4—*Continued*

NO.	SUBSTANCE	REFERENCE
LXXX	Structures not yet determined	
	B.P. Peroxide	(9)
	$C_{26}H_{22}O_3$	(13)
	$C_{24}H_{22}O_2Cl$	(8)
	$C_{24}H_{22}O_2Cl_2$	(16)
	$C_{24}H_{22}O_2Br_2$	(16)
	$C_{28}H_{22}O_2Br_6$	(19)

A few points of particular interest may be mentioned: 1,2,4,5-tetraphenylbenzene has been obtained from (a) anhydroacetonebenzil and tolane and (b) the dimer of 2,3,5-triphenylcyclopentadienone (which is another of the carbonyl bridge bimolecular products that dissociates) and phenylacetylene; this tetraphenylbenzene proved to be identical with the one formed by the action of phenylmagnesium bromide on hexabromobenzene (43). While the phenylacetylene might have added in two ways to give isomers, only the one hydrocarbon was produced. Dilthey also prepared 1,2,3,4-tetraphenyl-, hexaphenyl- and pentaphenyl-benzenes. The last-named hydrocarbon has also been obtained from tetracyclone and β -nitrostyrene; the addition product was not isolated since it lost carbon monoxide and nitrous acid so easily (6).

(ii) *Nitroso compounds*: A few aromatic nitroso compounds have been added to tetracyclone and phencyclone (44, 46), the $-N=O$ double bond resembling a $C=C$ linkage. No intermediate carbonyl bridge compound could be isolated, for decarbonylation occurred too easily, but there seems to be little doubt that it was an intermediate. The similarity in structure of the final product, the oxazine LXXXVI, to other decarbonylated dihydroaromatics is noteworthy.



(d) Unsaturated six-membered rings having lactone and anhydride bridges

Two other types of bridged six-membered rings are related in their behavior to the carbonyl bridge compounds; they are lactones and anhydrides.



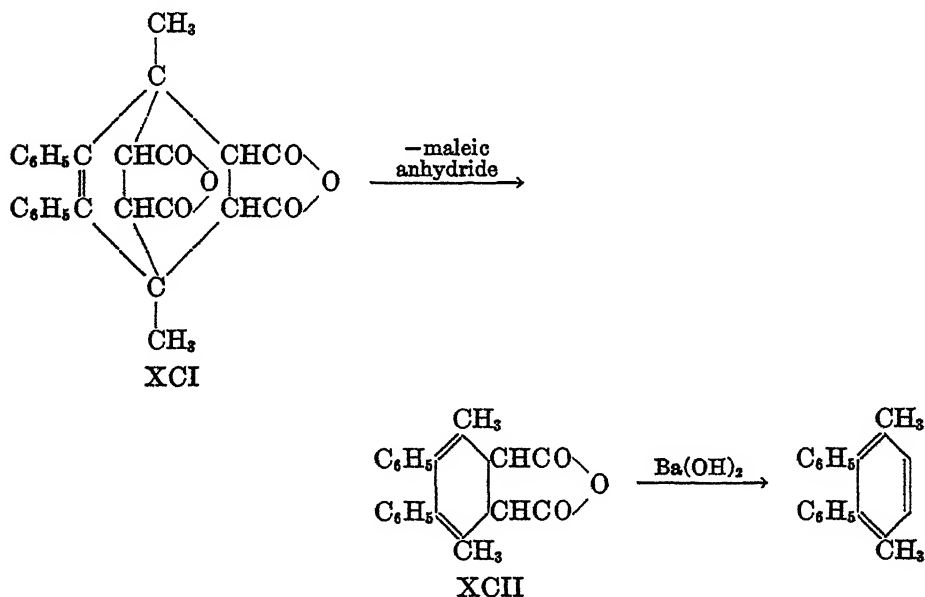
The lactones, which have a $-C(=O)-O-$ bridge between the 1- and 4-positions, are obtained by the application of the diene synthesis to pyrones (2, 36). If the synthesis is carried out at a moderate temperature, the desired addition product LXXXVII is formed, but at higher temperatures the bridge is lost as carbon dioxide; the expected dihydroaromatic substance (LXXXVIII) at once

All compounds formed by addition of maleic anhydride to the terpenes and polynuclear aromatic hydrocarbons will be omitted, since these have been summarized elsewhere (70). Those related to this work are collected in table 6.

The chemical reactions of the dianhydrides are those expected from their structures; they may be restricted somewhat on account of steric influences. The dianhydride XXXVII ($R = H$) can be esterified completely to give the fully alkylated ester (XC) of the tetracarboxylic acid (18).

The substituted dianhydride XXXVII ($R = CH_3$) titrates as a dibasic acid in the cold, the second ring opening only on warming (19).

When heated to elevated temperatures, the dianhydrides lose one equivalent of maleic anhydride; this is separable by distillation, but the residue, which is a dihydroaromatic (XCII), is often difficult to manipulate because of decomposition brought about by the heat. If barium hydroxide is previously mixed intimately with the dianhydride XCI, decarboxylation takes place in addition to the loss of the maleic anhydride, and an aromatic hydrocarbon is formed (12). This affords a source of certain highly arylated hydrocarbons not readily obtainable in other ways; e.g., 2,3-diphenyltoluene (22); 2,3-diphenyl-*p*-xylene (19); 2,3-diphenyl-*n*-amylbenzene (22).



IV. LOSS OF THE BRIDGE AND ITS RELATION TO THE DOUBLE-BOND RULE

In the previous pages, a summary of the behavior of a variety of closely related bridged compounds has been given. All these had as common features of structure (a) a six-membered ring containing an ethylenic linkage, (b) a bridge, and (c) a particular relation between the unsaturation and the bridge. As a common property, they all lost the bridge when heated, giving rise to a dihydroaromatic compound; the bridge appeared as a small molecule. While

TABLE 5

Unsaturated six-membered ring compounds with a lactone bridge

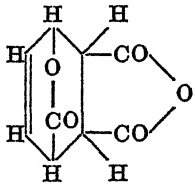
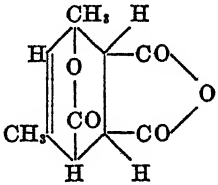
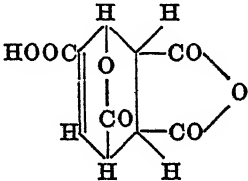
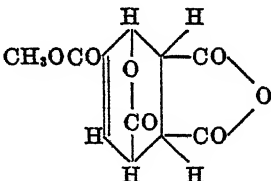
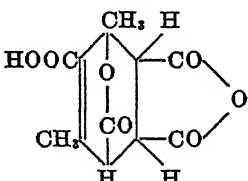
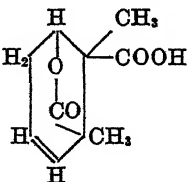
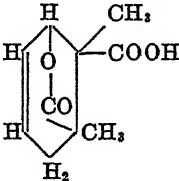
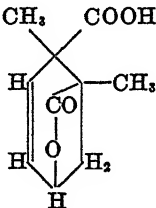
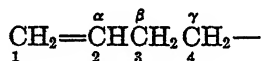
NO.	SUBSTANCE	REFERENCE
LXXXVII		(35)
		(35)
		(35)
		(35)
		(35)
		(83)

TABLE 5—Continued

NO.	SUBSTANCE	REFERENCE
		(83)
		(83)

this behavior was at first incorrectly associated with the nature of the bridge, it has become obvious with the accumulation of a large number of instances, that it is not a property of the bridge as such, but it is its relation to the unsaturated linkage that is responsible for this cleavage.

Staudinger (76) was the first to point out that in an unsaturated bond system, such as



the strength of the bonds between carbon atoms 3 and 4 (or β to the double bond) is less than that between 2 and 3, so that should scission occur, it would take place preferably at that point. This was independently enunciated by Hurd (59). In a paper on the dissociation of carbon bonds, Schmidt (74) drew attention to the double-bond rule—"the double bond between two carbon atoms strengthens the following single carbon bond and weakens the next following"—and cited seven instances. This statement is conveniently known as Schmidt's double-bond rule (52).

Using the ketone XXII in illustration, it will be noted that in the structural formula there are two sets of single bonds which are beta to the ethylenic linkage. Either of these would be likely to cleave according to the double-bond rule. If cleavage occurs in the direction indicated by the dashes, the result is tetracyclone and styrene—that is, the components have been regenerated, and the diene synthesis has been reversed. However, if the molecules break as indicated by the dots, carbon monoxide is split out, and pentaphenyldihydrobenzene is formed. Since the diene synthesis is reversible, while the loss of carbon monoxide is irreversible, the reaction will run to completion in this direction (namely, elimination of the bridge). (See page 257.)

TABLE 6

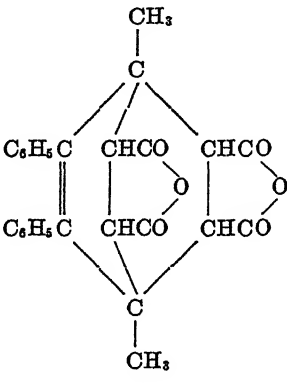
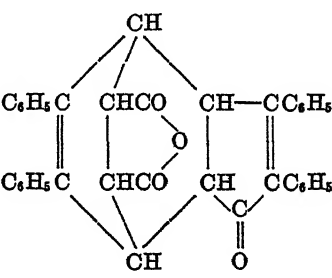
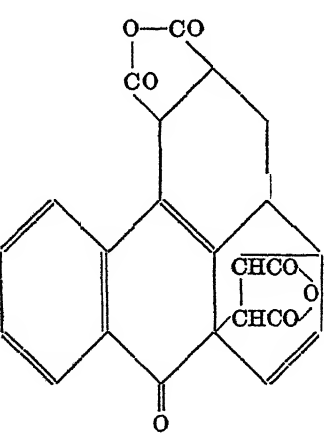
Six-membered ring compounds with an anhydride bridge

NO.	SUBSTANCE	REFERENCE
LXXXIX		(36)
		(36)
		(33)
		(36)

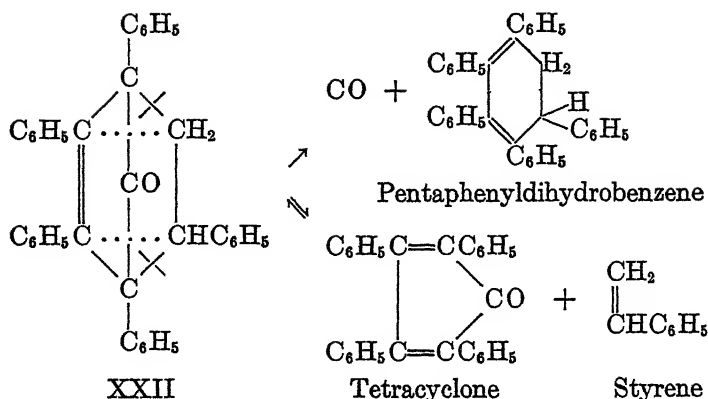
TABLE 6—Continued

NO.	SUBSTANCE	REFERENCE
XXXVII; R = H		(36)
		(15, 18)
LXVII; R = CH ₃		(22)
LXVIII; R = <i>n</i> -C ₈ H ₁₇		(22)

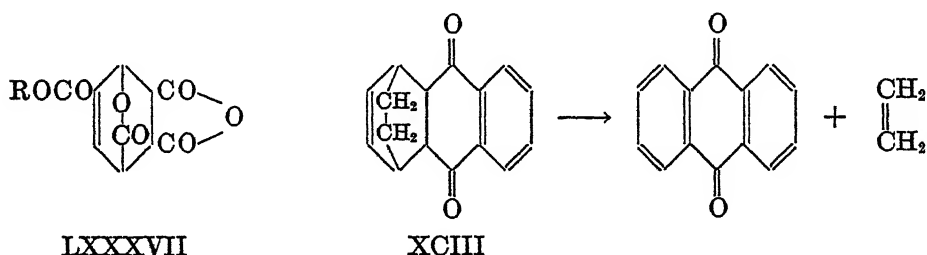
TABLE 6—*Concluded*

NO.	SUBSTANCE	REFERENCE
XCI		(19)
		(18)
		(34)

Taking the double-bond rule into account, it is thus possible to predict what products of heating are to be expected in similar instances. The lactone bridge ester LXXXVII would lose carbon dioxide, and the dianhydrides would lose maleic anhydride. On experiment these reactions were found to take place.

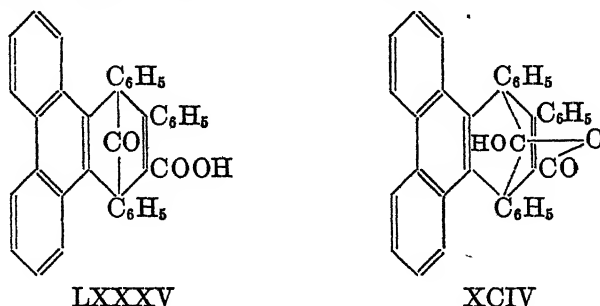


A considerable number of endoethylene bridge compounds of the type XCIII, formed by the diene synthesis, are likewise unstable to heat; ethylene is lost and a dihydroaromatic substance obtained (35). It should furthermore be noted



that in these bridged compounds the ethylenic linkage affects *both* bonds holding the bridge. This was not the case with the large-membered ring ketones VIII and XII considered in the first part of this paper. This difference in linkage affords a partial explanation of why the bridge was not lost when they were submitted to high-temperature distillation. Finally, saturated six-membered bridged rings should be stable when heated. The terpene ketones are, for they can be distilled.

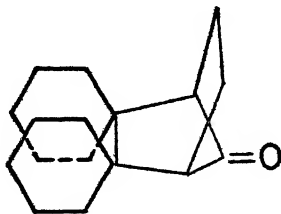
In the case of acetylenic addends, the bridge bonds come under the influence of two double bonds, i.e., they are in the β -position; thus, they will be weakened to a much greater degree, and it would be expected that the bridge would be



lost more easily, as is, indeed, found to occur. Conversely, it would be very difficult or impossible to isolate the bridged compound. The only exception, the acid LXXXV, can be accounted for by assuming that the carboxyl group has formed a ring with the bridge. On account of the observation that this acid did not dissolve as expected, even in alcoholic alkali, Dilthey (40) devised such a ring structure (XCIV).

It may be noted that practically all of the carbonyl bridge compounds examined have phenyl groups at the ends of the ethylenic linkage. No method has yet been discovered for reducing this double bond, so that comparable saturated carbonyl bridge compounds are unknown. When the simplest indene having a carbonyl bridge (XLIX) is reduced by hydrogen in the presence of the very active platinum oxide catalyst, only the double bond in the side chain is affected (56); the indane L results.

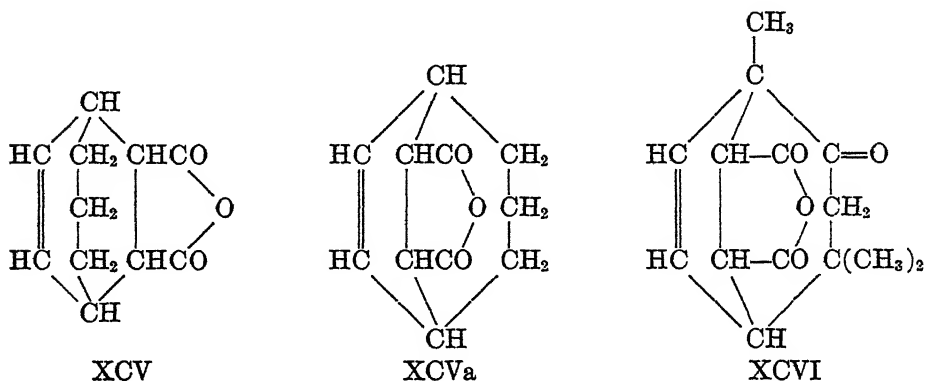
It is believed that in order for reduction to take place (at least in aromatic nuclei) the molecule to be reduced must be adsorbed on the surface of the catalyst in a planar configuration (92). Thus, the catalytic reduction of a hindered biphenyl derivative, in which rotation about the bond connecting the two phenyl groups was impossible, could not be accomplished even by the most drastic conditions of temperature and pressure (93). A similar situation is met in the compound under discussion. The two phenyl groups attached to the doubly bound carbons cannot lie in the plane of the double bond (*cf.* *o*-terphenyl (91)). This would offer considerable hindrance toward the double bond being adsorbed on any plane catalytic surface. In addition, the carbonyl group not only offers hindrance to adsorption but also forces the cyclohexene ring into the "bed"



form, such that it also hinders adsorption. Thus, assuming the correctness of the adsorption hypothesis, it would appear that the reduction by catalytic methods of the ethylenic double bond bearing two phenyl groups is most unlikely to take place.

Summarizing, the behavior of the bridged molecules in a six-membered ring having an ethylenic linkage is in accord with and explicable in the light of the double-bond rule. *The behavior of the bridge is dependent upon its relation to the double bond, and not on the nature of the atoms forming the bridge.* As Norton states, it is to be emphasized "that the bridge, as such, does not confer special properties on the atoms included in the bridge, although the natural human tendency appears to be that one will tend to place the more reactive portion of the molecule in the bridge on drawing the structure of such a bridged compound" (70).

Cycloheptadiene and eucarvone form addition products XCV and XCVI with maleic anhydride (3, 60, 85). Each of these substances can be considered either as a six-membered ring with a three-atom-chain bridge, or as a seven-membered ring with a two-atom-chain bridge (XCVa). Little is known of their chemical behavior, probably on account of the small amounts available.



In the light of the foregoing evidence, it would be expected that on being heated these substances would either (a) dissociate into their components or (b) undergo cleavage of the three-carbon-atom system, analogous to the bridge in the six-carbon-atom types. While it is recorded in the literature (2) that XCV dissociates into its components, no experimental details are given.

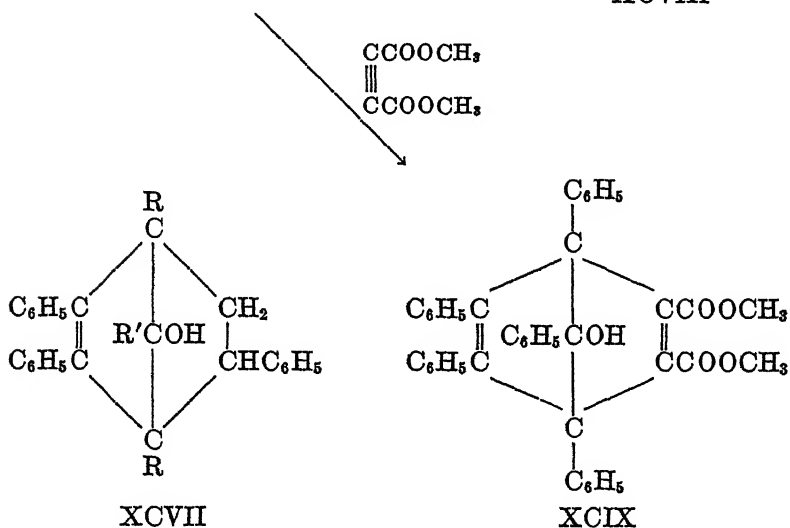
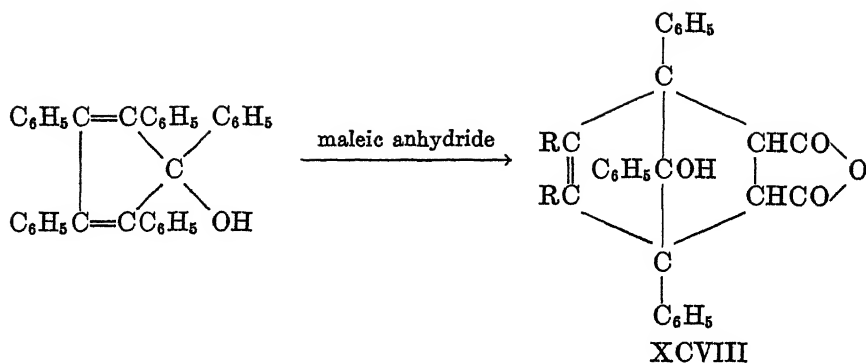
V. CARBINOL BRIDGE COMPOUNDS

Since the substances having a carbinol bridge $\text{R}-\text{C}-\text{OH}$ have all been prepared in connection with the carbonyl bridge work and have certain comparable properties, it is proper that they be included in any survey covering the entire field.

Only a small number of this type (XCVII, XCVIII, XCIX) of substance is known. They have all been obtained either (1) by the action of a Grignard reagent on a carbonyl bridge compound (19, 20) or (2) by a direct diene synthesis (20, 21). Those in the six-membered-ring series, secured by the first procedure, can only be obtained if there is no hydrogen alpha to the bridge carbonyl. The only known example of a substance (XIII) in which the carbinol bridge is across an eight-membered ring has already been described (page 214); it had no unusual properties (14).

The carbinol bridge compounds are collected in table 7.

Since this type of substance was prepared solely to learn its behavior when heated, there are only a few other known facts. The hydroxyl group was replaced by chlorine when the carbinol was treated with acetyl chloride (19); the chloride C lost hydrogen chloride when heated, but the nature of the product has not been determined.



The behavior of the carbinols on heating is interesting. A variety of products is obtained, most of which are cleavage fragments. Usually it appears that the molecule has dissociated into a diene and an ethylenic compound—a reverse diene synthesis. The diene component then undergoes further changes.

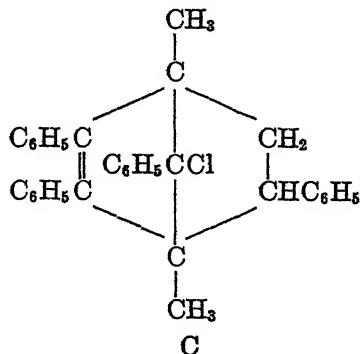


TABLE 7

Six-membered ring compounds with a carbinol bridge

NO.	SUBSTANCE	REFERENCE
CI		(20)
XCVIII; R = 4-BrC ₆ H ₄		(21)
XCVII; R, R' = CH ₃		(19)
XCVII; R = CH ₃ R' = C ₆ H ₅		(19)

TABLE 7—Continued

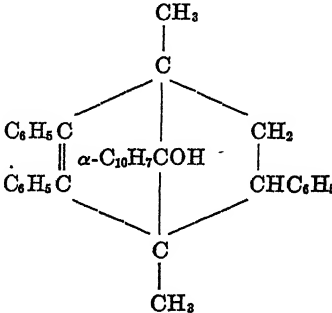
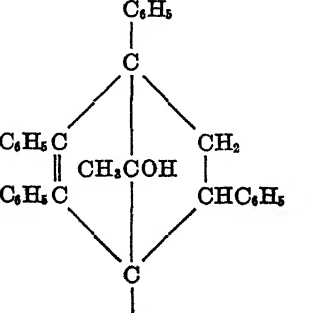
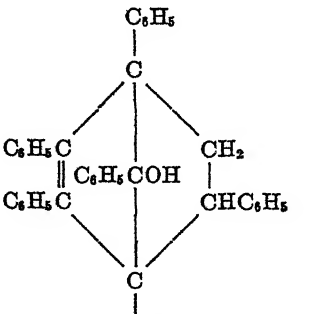
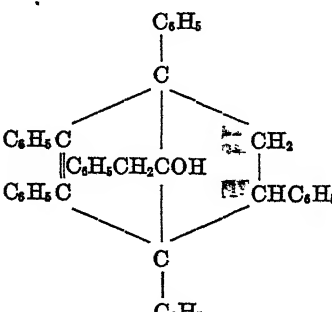
NO.	SUBSTANCE	REFERENCE
XCVII; R = CH ₃ R' = α -C ₁₀ H ₇		(19)
XCVII; R = C ₆ H ₅ R' = CH ₃		(20)
CV		(20)
CVI		(20)

TABLE 7—*Concluded*

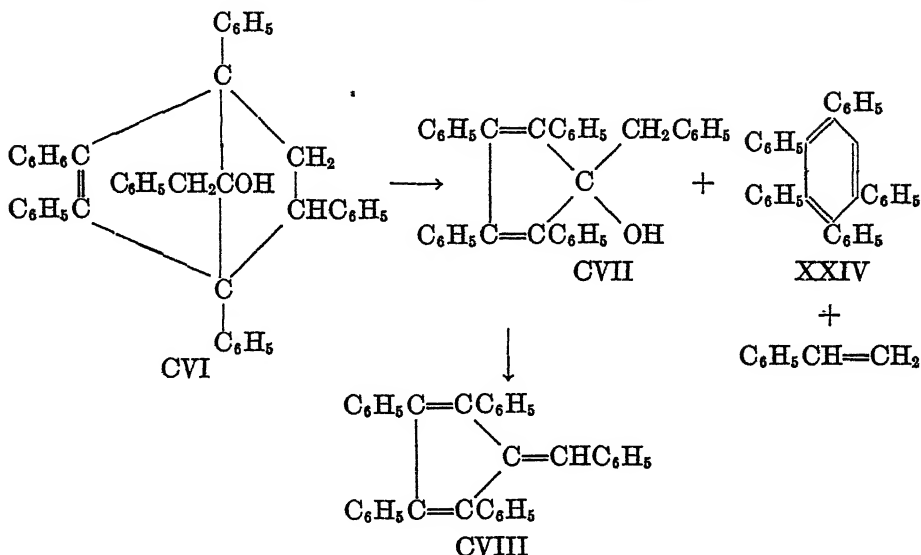
NO.	SUBSTANCE	REFERENCE
XCIX		(20)
LXIII		(8)

Pentaphenylcyclopentadienol (Ziegler's carbinol) and maleic anhydride gave an addition product having a carbinol bridge, CI (XCVIII; $R = C_6H_5$) (20). When this addition product was heated *in vacuo*, a complex mixture was formed; among the easily recognized products were water, maleic anhydride, benzaldehyde, and pentaphenylcyclopentadiene (CII). There were also two isomers, CIII and CIV, which are also isomers of the carbinol. When Ziegler's carbinol was heated by itself under the same conditions, it gave the two isomeric substances, CIII and CIV, and pentaphenylcyclopentadiene. It seems obvious that the complex bridge compound must have dissociated into its components; a secondary reaction of the regenerated Ziegler's carbinol then gave rise to the cyclopentene derivatives.

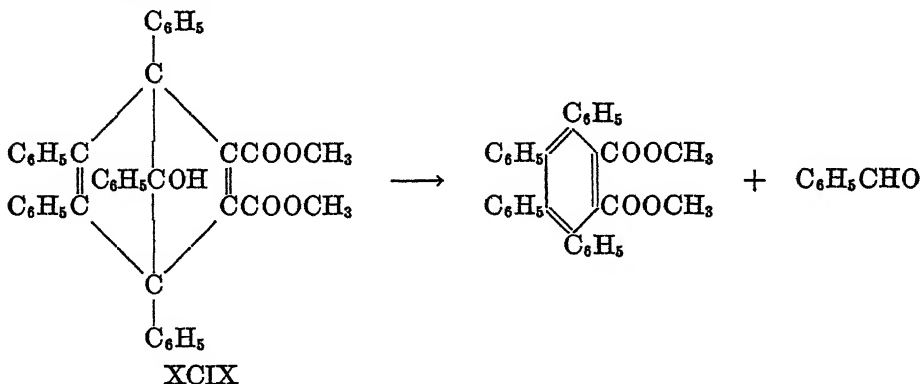
The pair of isomers CIII and CIV are interconvertible; they are formed from Ziegler's carbinol by a 1,3-rearrangement of a phenyl group (20). A brominated homolog of Ziegler's carbinol showed a similar behavior (21).

When the carbinol CV (XCVII; $R, R' = C_6H_5$) was heated, there were formed the same substances that resulted from Ziegler's carbinol alone, plus styrene and an oxygen-containing substance, $C_{36}H_{28}O$, the structure of which has not yet

does not involve the bridge carbon or aryl group attached thereto. It was not possible to determine the fate of the bridge in this instance (20).



In order to have a more favorable example in which one mode of cleavage would be expected to predominate, the addition product (XCIX) of Ziegler's carbinol and acetylenedicarboxylic ester was prepared (20). This substance would be expected to lose the bridge very easily, because it is attached to carbon atoms which are located beta to two sets of double bonds, both of which favor cleavage of the same single bonds. This expectation was realized, for the reaction was clean, giving but two products, benzaldehyde and tetraphenylphthalic ester.



In this instance, the fate of the bridge is clear—it appears as benzaldehyde.

It should be pointed out that it requires a much higher temperature to decompose the carbinol bridge compounds than is needed for the other types discussed. Presumably this is accounted for in the other types by the formation of substances

that require no rearrangement of atoms or groups to be stable molecules. In the case of the carbinols, the first cleavage product is an unstable form of an aldehyde. Since this is not a normal product, it requires a greater amount of energy to bring about the cleavage. Thus, the isolation of the ester XCIX, formed with an acetylenic addend, is possible.

In summation: When six-membered cyclic substances having a carbinol bridge and one ethylenic linkage are heated, they resemble other similarly constituted compounds, differing only in the nature of the bridges. The molecule decomposes in two ways: either it dissociates into its components, which may undergo further changes, or it loses the bridge and gives a complex aromatic compound or substances closely related to it. The bridge appears as an aldehyde, which can be isolated as such in favorable circumstances.

When the carbinol bridge compound contains two ethylenic linkages, but one mode of decomposition occurs—that in which the bridge is eliminated.

The enthusiastic collaboration of my former graduate students at McGill University and the valuable aid of my colleagues in the Synthetic Organic Research Laboratory is hereby gratefully acknowledged.

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THE CHEMISTRY OF CINNOLINES

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CONTENTS

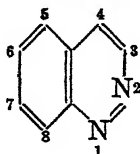
I. Introduction.....	269
II. Relation to other binitrogen heterocycles.....	269
III. Synthesis of cinnolines.....	270
A. General methods.....	270
1. From <i>o</i> -aminophenylpropionic acids ..	270
2. From <i>o</i> -aminophenylethylenes....	270
3. From <i>o</i> -aminoacetophenones.....	272
B. Special methods.....	272
1. From hydrazine and substituted hydrazines.....	272
2. From diazo compounds.....	276
3. From styrene by a Diels-Alder condensation	278
4. By reduction of 2,2'-dinitrobiphenyls.....	278
IV. Properties of cinnolines	280
A. Physical properties.....	280
B. Chemical properties	280
1. Salt formation	280
2. Oxidation	280
3. Reduction.....	282
4. Replacement reactions.....	284
V. Summary.....	284
VI. References	285

I. INTRODUCTION

The discovery of cinnoline dates from 1883, but the compound and its derivatives have not received the attention accorded related heterocyclic nitrogen compounds. A number of substituted cinnolines have been described as useful dyes (22, 23, 24, 42, 43). No suggestion has yet appeared in the literature as to the possible utility of cinnoline compounds as medicinals or as reagents for analytical chemistry.

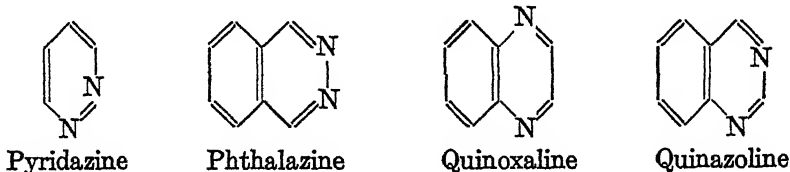
II. RELATION TO OTHER BINITROGEN HETEROCYCLES

Cinnoline is a heterocyclic binuclear base containing two vicinal nitrogen atoms. The numbering system follows that in quinoline. Cinnoline is related



Cinnoline

to pyridazine in that it is benzo[*c*]pyridazine or 3,4-benzopyridazine. It is the least well known of the family of condensed bicyclic aromatic compounds having



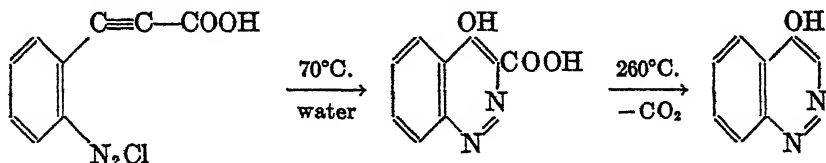
two nitrogen atoms in the same ring. The other members of the family are phthalazine, quinoxaline, and quinazoline.

III. SYNTHESIS OF CINNOLINES

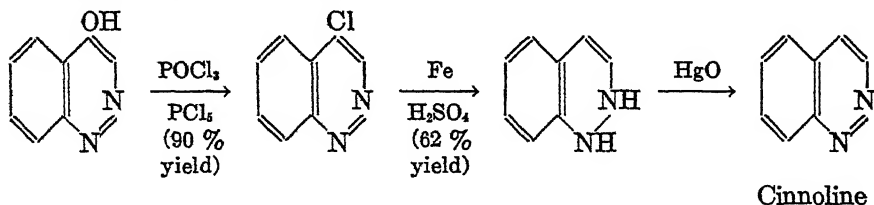
A. GENERAL METHODS

1. From *o*-aminophenylpropionic acids

The first synthesis of cinnoline was reported in 1883 by von Richter (32), who did not obtain the new compound in sufficiently pure condition for determination of its physical properties or for elementary analysis. The diazonium chloride obtained from *o*-aminophenylpropionic acid was heated in water solution at 70°C. Cooling caused the separation of 4-hydroxycinnoline-3-carboxylic acid in quantitative yield. When this acid was heated above its melting point, carbon dioxide was liberated and 4-hydroxycinnoline was formed in nearly theoretical yield. Distillation of 4-hydroxycinnoline with zinc dust furnished a small amount of a basic oil, which was assumed to be cinnoline.

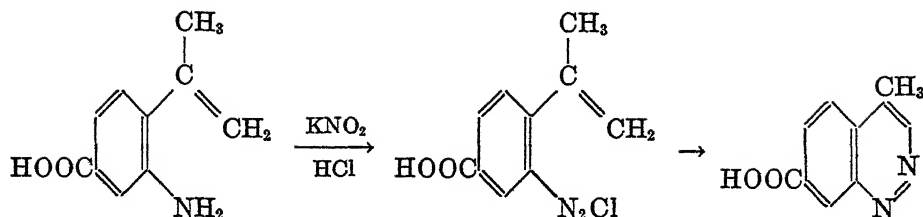


This preparation of 4-hydroxycinnoline was repeated by Busch and Klett (11), although in less satisfactory yield than that reported by von Richter. The 4-hydroxycinnoline was converted successfully to cinnoline *via* the 4-chloro compound by Busch and Rast (12).

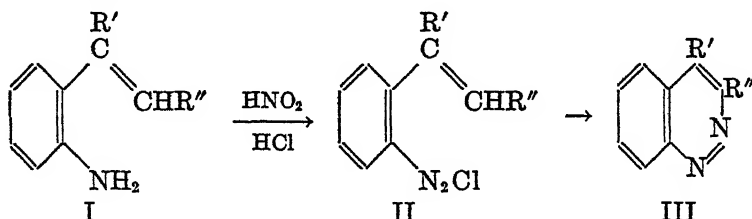


2. From *o*-aminophenylethylenes

When diazonium salts prepared from certain *o*-aminophenylethylenes are allowed to stand, cinnolines are formed. When diazotized 3-amino-4-isopropenylbenzoic acid was allowed to stand at room temperature, it was found by Widman (53, 54) to undergo ring closure to 4-methylcinnoline-7-carboxylic acid.



This method was extended by Stoermer, Gaus, and Fincke (44, 45). Diazotization of the substituted *o*-aminophenylethylene was followed by cyclization, which usually occurred spontaneously at room temperature in nearly quantitative yield.



Stoermer obtained substituted cinnolines when R' was phenyl, *p*-tolyl, or *p*-anisyl and R'' was hydrogen, and when R' was phenyl and R'' was methyl.

TABLE 1

*Influence of substituents R' and R'' in the formation of cinnolines from *o*-aminophenylethylenes*

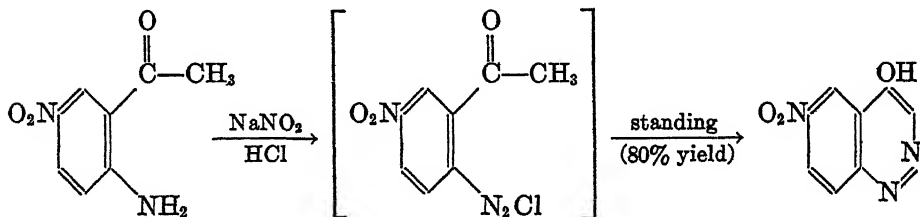
R'	R''	RESULT
COOH	C_6H_5	(Pschorr reaction)
COOH	<i>m</i> - $CH_3C_6H_4$	(Pschorr reaction)
H	C_6H_5 (<i>cis</i>)	(Pschorr reaction)
H	COOH	(No cinnoline formation)
H	$COOC_2H_5$	(No cinnoline formation)
H	CN	(No cinnoline formation)
C_6H_5	H	4-Phenylcinnoline
C_6H_5	CH_3	3-Methyl-4-phenylcinnoline
C_6H_5	C_6H_5 (<i>cis</i>)	3,4-Diphenylcinnoline
C_6H_5	C_6H_5 (<i>trans</i>)	3,4-Diphenylcinnoline
C_6H_5	$C_6H_5CH_2$	3-Benzyl-4-phenylcinnoline
C_6H_5	α - $C_{10}H_7$	3-(1'-Naphthyl)-4-phenylcinnoline

When R' was phenyl and R'' was bromine, diazotization in hydrochloric acid solution produced 4-phenylcinnoline in 22 per cent yield rather than the expected bromophenylcinnoline. Stoermer deduced, from the fact that *o*-aminocinnamic acid did not yield a cinnoline, that a negative group (R'') on the β -ethylenic carbon atom of the *o*-aminophenylethylene inhibits the cyclization. In a recent report of original experiments, correlated with existing data on the synthesis of cinnolines by the Widman-Stoermer reaction, Simpson (40, 41) has indicated the limitations of this method of cyclization ($I \rightarrow II \rightarrow III$). It will be seen in table 1 that the nature of the substituents R' and R'' has a strong directing effect

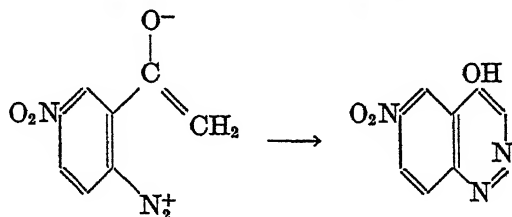
upon the course of the reaction. The available data lead to the conclusion that cinnoline formation does not occur when R'' is aryl or another negative group and R' is either hydrogen or carboxyl. When R' is a phenyl or a substituted phenyl group, cinnoline formation is favored in spite of the presence of an aryl group, R'' , on the β -carbon atom. 3,4-Diphenylcinnoline was obtained in quantitative yield from either *cis*- or *trans*- α -(*o*-aminophenyl)- α , β -diphenylethylene; therefore, in this case at least, the cyclization is independent of spatial configuration.

3. From *o*-aminoacetophenones

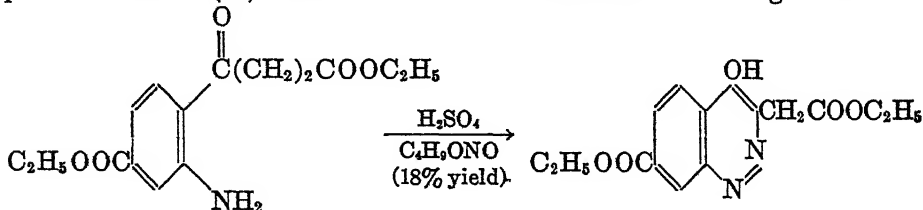
A related method, which would appear to be general for the synthesis of cinnolines, involves the diazotization of an *o*-aminoacetophenone. Borsche and Herbert (8) diazotized 2-amino-5-nitroacetophenone and allowed the diazonium salt solution to stand at room temperature. The product, 4-hydroxy-6-nitrocinnoline, separated in 80 per cent yield.



The reaction probably involves an intramolecular coupling of the diazonium



cation with the enolate anion. Such a mechanism would be similar to the coupling of an aryldiazonium ion with a phenoxide ion to form an azo compound. Koelsch (26) carried out a similar diazotization and ring closure:

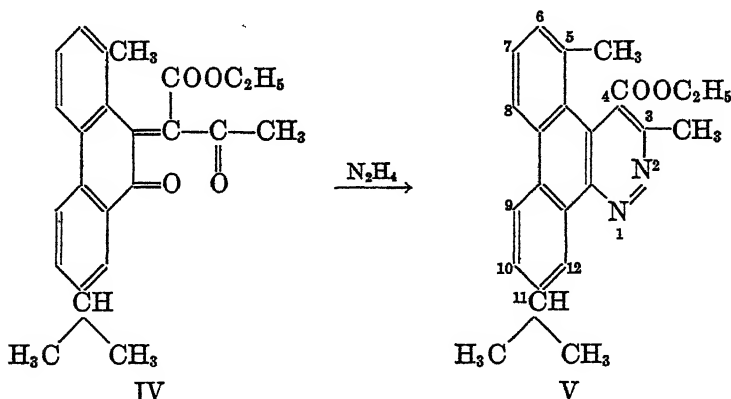


B. SPECIAL METHODS

1. From hydrazine and substituted hydrazines

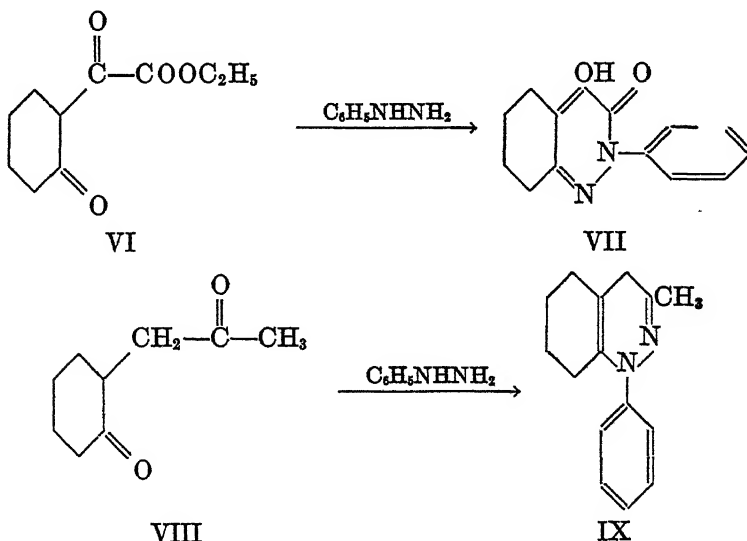
The reaction of a dicarbonyl compound with hydrazine to form an azine is well known. This reaction has been used to advantage by a number of workers

for the preparation of cinnolines from certain 1,4-dicarbonyl compounds. An example is provided by Heiduschka and Khudadad (21), who synthesized ethyl 3,5-dimethyl-11-isopropyldibenzo[*f,h*]cinnoline-4-carboxylate (V) by the condensation of "retoxyleneacetoacetic ester" (one possible formula, IV, is given) with hydrazine.



Further examples are found in table 2. Although some of the products are named on the basis of pyridazine or phthalazine, all may equally well be regarded as substituted cinnolines. Only highly substituted polynuclear cinnoline derivatives have been prepared in this manner.

Just as hydrazine has been used with certain 1,4-dicarbonyl compounds for cinnoline formation, so phenylhydrazine has been utilized (6, 18). Reaction of phenylhydrazine with ethyl cyclohexanoneoxalate (VI) gave 5,6,7,8-tetrahydro-4-hydroxy-2-phenyl-3(2)-cinnolone (VII). Reaction of phenylhydrazine with acetonylcyclohexanone (VIII) furnished 1,4,5,6,7,8-hexahydro-3-methyl-1-phenylcinnoline (IX).



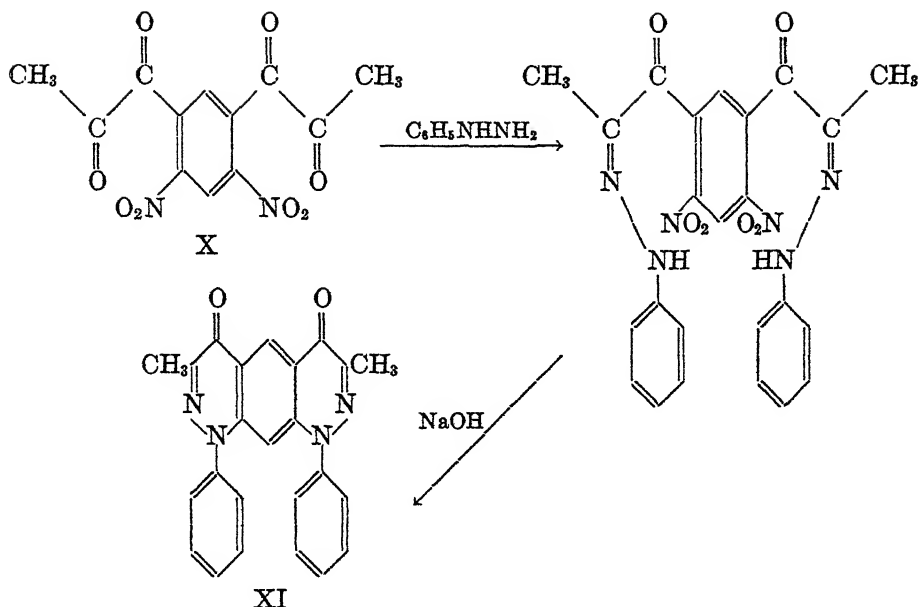
Closely related to these reactions with phenylhydrazine is the synthesis of 3,7-dimethyl-1,9-diphenylpyridazo[4,3-*g*]cinnoline-4,6(1,9)-dione (XI) by Ruggli and Straub (33).

TABLE 2

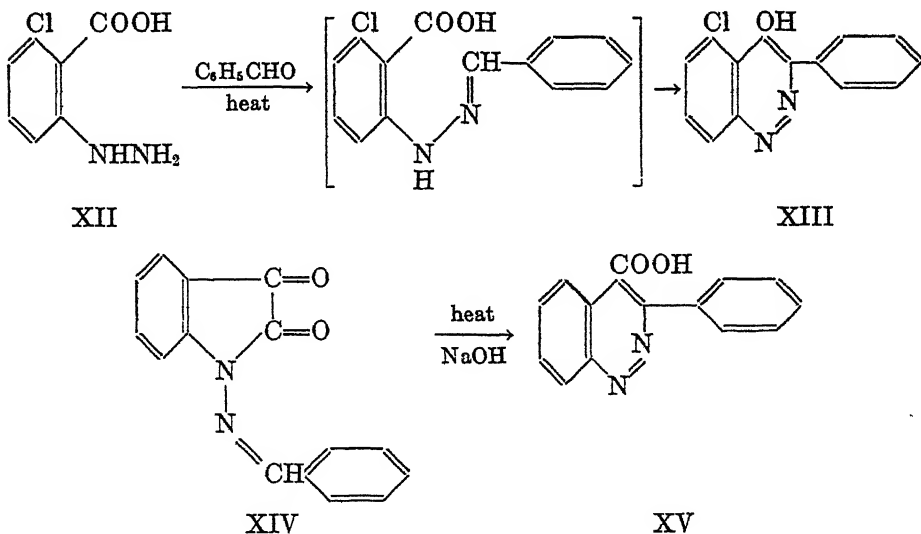
Formation of cinnolines and related compounds by condensation of diketones with hydrazine

DIKETONE	PRODUCT	REFERENCE
3,9-Dibenzoylperylene-4,10-quinone.	3,10-Diphenylperyl[3,5- <i>cd</i> ,9,10- <i>c'd'</i>]dipyridazine	(7)
Ethyl γ -phenyl- α -phenacylacetoacetate	3-Phenylbenzo[<i>g</i>]cinnolin-5(10)-one	(9)
1,2,3,4 - Tetrahydro - 1 - keto - 3 - phenyl-2-naphthaleneacetic acid...	1,2,5,6-Tetrahydro-5-phenylbenzo[<i>h</i>]cinnolin-3(4)-one	(10)
Dibenzo[<i>a,h</i>]phenanthrene - 5,6,7,12-tetrone.....	Dinaphtho[1,2,3- <i>de</i> ,1',2',3'- <i>ij</i>]phthalazine-7,14-dione	(13)
Dibenzo[<i>c,m</i>]pentaphene - 5,8,15,16-tetrone.....	Diphenanthro[1,2,3- <i>de</i> ,3',2',1'- <i>ij</i>]phthalazine-5,8-dione	(14)
11-Methylbenzo[<i>c</i>]pentaphene-5,8,13,14-tetrone.....	11-Methylnaphtho[1,2,3- <i>de</i>]phenanthro[3',2',1'- <i>ij</i>]-phthalazine-5,8-dione	(14)
2,10-Dimethyldibenzo[<i>b,h</i>]phenanthrene-5,6,13,14-tetrone.....	2,10-Dimethyldinaphtho[1,2,3- <i>de</i> ,3',2',1'- <i>ij</i>]phthalazine-5,8-dione	(15)
Retoxyleneacetoacetic ester.....	Ethyl 3,5-dimethyl-11-isopropylidibenzo[<i>f,h</i>]cinnoline-4-carboxylate	(21)
Triphthaloylbenzene.....	Trinaphthyleno[5,6,11,12]dipyridazine-17,18-dione	(30)
Dibenzo[<i>b,h</i>]phenanthrene-5,8,13,14-tetrone-3-carboxylic acid.....	Dinaphtho[1,2,3- <i>de</i> ,3',2',1'- <i>ij</i>]phthalazine-5,8-dione-3-carboxylic acid	(37)
Dibenzo[<i>b,h</i>]phenanthrene-5,8,13,14-tetrone.....	Dinaphtho[1,2,3- <i>de</i> ,3',2',1'- <i>ij</i>]phthalazine-5,8-dione	(38)
1-Benzoyl-5-chloroanthraquinone....	8-Chloro-3-phenylnaphtho[1,2,3- <i>de</i>]phthalazin-7-one	(42)
1-Amino-5-benzoylanthraquinone...	8-Amino-3-phenylnaphtho[1,2,3- <i>de</i>]phthalazin-7-one	(43)
1-Propionylanthraquinone.....	3-Ethynaphtho[1,2,3- <i>de</i>]phthalazin-7-one	(52)

There are two examples of the preparation of cinnolines from substituted phenylhydrazones of benzaldehyde. 5-Chloro-4-hydroxy-3-phenylcinnoline (XIII) was obtained in low yield by Pfannstiel and Janecke (29), and 3-phenyl-

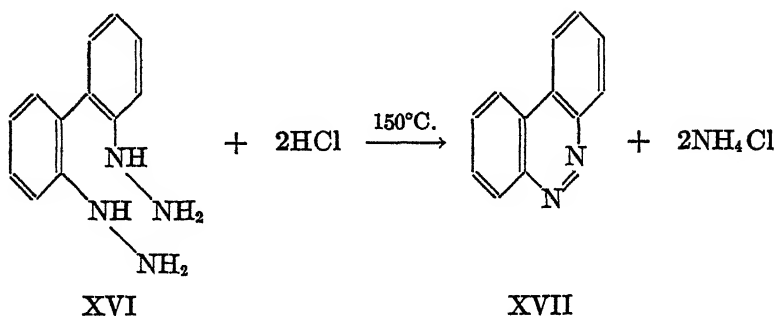


cinnoline-4-carboxylic acid (XV) was considered to be the product obtained by Stollé and Becker (46) in the following reactions:



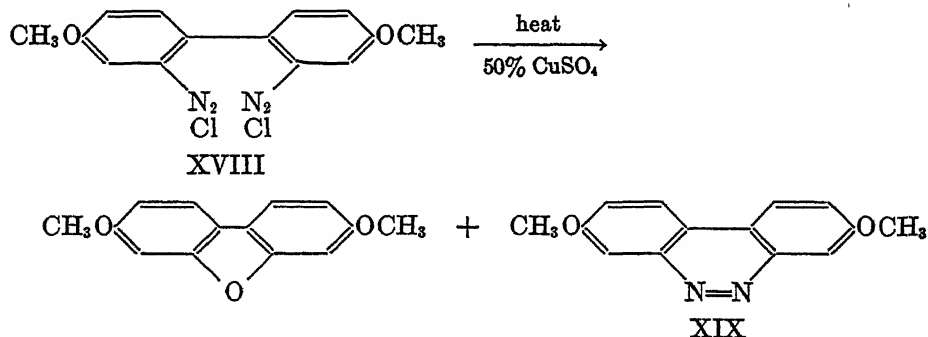
A dihydrazino compound has also been used for the preparation of a cinnoline. When 2,2'-dihydrazinobiphenyl (XVI) was heated with hydrochloric acid under pressure, Täuber (50) was able to obtain benzo[c]cinnoline (XVII) in quantitative yield. The reaction is parallel to the formation of carbazole by the

heating of 2,2'-diaminobiphenyl with acid. Täuber likewise obtained benzo[c]cinnoline by heating the diacetyl derivative of XVI.



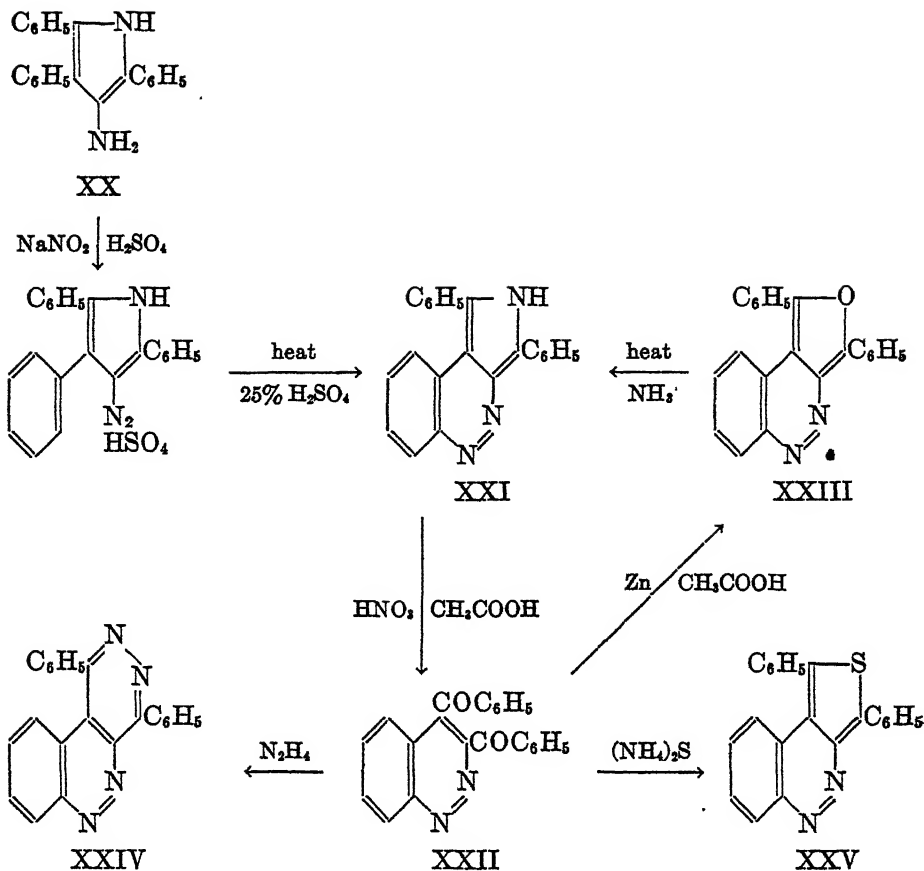
2. From diazo compounds

Benzo[c]cinnolines have been prepared from tetrazonium salts. In 1935, Hata, Tatematsu, and Kubota (20) obtained 3,8-dimethoxybenzo[c]cinnoline (XIX) as a by-product in their synthesis of 2,7-dimethoxydiphenylene oxide by treatment of tetrazotized 2,2'-diamino-4,4'-dimethoxybiphenyl (XVIII) with copper sulfate.

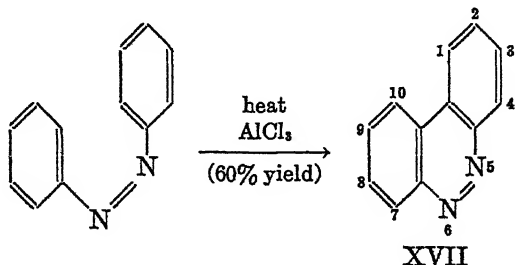


Later, Sandin and Cairns (35) obtained a 45 per cent yield of benzo[c]cinnoline by treatment of tetrazotized 2,2'-diaminobiphenyl with arsenious oxide in sodium carbonate solution. 3,8-Dimethylbenzo[c]cinnoline was prepared in a like manner from 2,2'-diamino-4,4'-dimethylbiphenyl.

When 4-amino-2,3,5-triphenylpyrrole (XX) was diazotized, it was found that the resulting diazonium salt could be converted to a cinnoline (XXI) by heating with dilute sulfuric acid. Angelico (1, 2, 3, 4, 5) used this diazonium salt for the preparation of several highly substituted cinnoline compounds: 1,3-diphenylpyrrolo[3,4-c]cinnoline (XXI), 3,4-dibenzoylcinnoline (XXII), 1,3-diphenylfuro[3,4-c]cinnoline (XXIII), 1,4-diphenylpyridazo[4,5-c]cinnoline (XXIV), and 1,3-diphenylthieno[3,4-c]cinnoline (XXV). Interconversion of these cinnolines was brought about by well-known reactions.



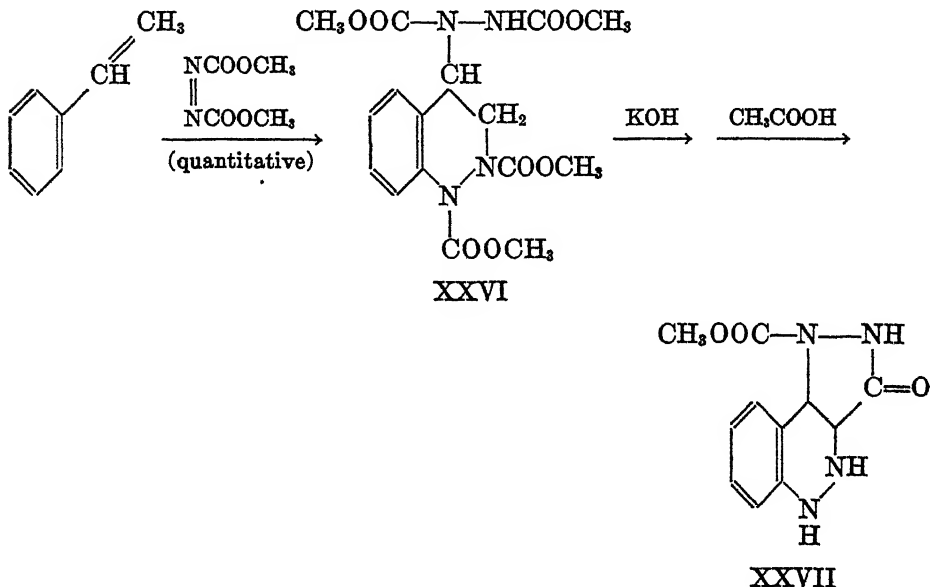
The conversion of a few azo compounds to cinnolines has been reported. The preparation of benzo[*c*]cinnoline (XVII) has been described in a patent (22) which calls for the fusion of azobenzene with aluminum chloride



and sodium chloride at 120°C. 3,8-Dimethylbenzo[*c*]cinnoline was similarly prepared from *m,m'*-azobistoluene at 100°C. in 25 per cent yield; 3,8-tetramethyldiaminobenzo[*c*]cinnoline, from *m,m'*-azobisdimethylaniline at 100°C. in 40 per cent yield.

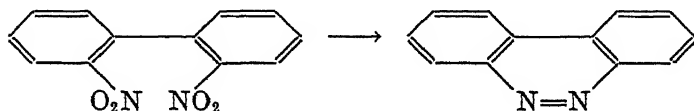
3. From styrene by a Diels-Alder condensation

The addition of dimethyl azodicarboxylate to styrene provides the only example of cinnoline (XXVI, XXVII) formation by means of a Diels-Alder reaction (16). α -Methylstyrene, propenylbenzene, and stilbene failed to give cinnoline-type compounds when treated with dimethyl azodicarboxylate.



4. By reduction of 2,2'-dinitrobiphenyls

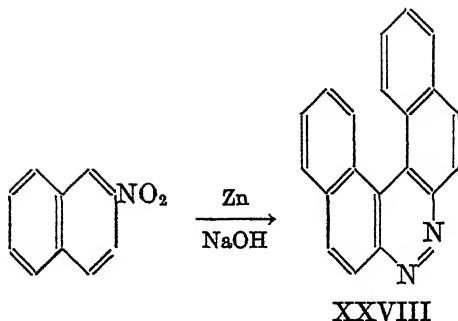
A number of workers have prepared substituted benzo[c]cinnolines by reduction of the correspondingly substituted 2,2'-dinitrobiphenyls. The reaction is parallel to the preparation of azobenzene by the reduction of nitrobenzene and is here illustrated for the production of benzo[c]cinnoline itself:



Examples of the production of substituted benzo[c]cinnolines by this method are found in table 3. Best yields are usually obtained by electrolytic reduction. Meisenheimer and Witte (27) prepared benzo[f]naphtho[2,1-c]cinnoline (XXVIII) by a somewhat similar reaction, the reduction of 2-nitronaphthalene with zinc and alcoholic sodium hydroxide. The cinnoline (XXVIII) was obtained in poor yield along with 2,2'-azonaphthalene and 2,2'-diamino-1,1-binaphthyl.

TABLE 3
Preparation of benzo[c]cinnolines by reduction of 2,2'-dinitrobiphenyls

BIPHENYL	REDUCING AGENT	BENZO[c]CINNOLINE	YIELD	REFER- ENCE
			<i>per cent</i>	
2,2'-Dinitrobiphenyl.....	Na(Hg) + CH ₃ OH	Benzo[c]cinnoline	55	(47)
	Na ₂ S, then $\begin{cases} \text{SnCl}_2 \\ \text{HCl} \end{cases}$	Benzo[c]cinnoline		(51, (17)
	Electrolytic	Benzo[c]cinnoline	95	(56)
4,4'-Diamino-2,2'-dinitro- biphenyl.....	Na(Hg) + CH ₃ OH	3,8-Diaminobenzo[c]- cinnoline		(47)
	Electrolytic	3,8-Diaminobenzo[c]- cinnoline	80	(51)
4,4'-Diamino-5,5'-dimeth- oxy-2,2'-dinitrobiphenyl....	Electrolytic	3,8-Diamino-2,9-di- methoxybenzo[c]- cinnoline	60	(51)
2,2'-Dinitro-4,4'-biphenyl- dicarboxylic acid.....	Zn + NH ₃	Benzo[c]cinnoline-3,8- dicarboxylic acid		(23)
4,4'-Difluoro-5,5'-dimethyl- 2,2'-dinitrobiphenyl.....	Na(Hg) + CH ₃ OH	3,8-Difluoro-2,9-di- methylbenzo[c]cin- noline	75	(36)
4,4'-Dimethyl-2,2'-dinitro- biphenyl.....	Electrolytic	3,8-Dimethylbenzo[c]- cinnoline		(51)
6,6'-Dimethyl-2,2'-dinitro- biphenyl.....	Na(Hg) + CH ₃ OH	1,10-Dimethylbenzo[c]- cinnoline		(25)
	Electrolytic	1,10-Dimethylbenzo[c]- cinnoline	62	(55)
	Na ₂ S, then $\begin{cases} \text{SnCl}_2 \\ \text{HCl} \end{cases}$	1,10-Dimethylbenzo[c]- cinnoline		(34)
2,2'-Dinitro-4,4'-tetraethyl- aminobiphenyl.....	Electrolytic	3,8-Tetraethylamino- benzo[c]cinnoline	50	(51)
2,2'-Dinitro-4,4'-tetra- methylaminobiphenyl.....	Electrolytic	3,8-Tetramethyl- aminobenzo[c]cin- noline	56	(51)
	Na ₂ S, then elec- trolysis	3,8-Tetramethyl- aminobenzo[c]cin- noline	54	(51)
	Na ₂ S, then $\begin{cases} \text{SnCl}_2 \\ \text{HCl} \end{cases}$	3,8-Tetramethyl- aminobenzo[c]cin- noline	68	(51)



IV. PROPERTIES OF CINNOLINES

A. PHYSICAL PROPERTIES

Cinnoline crystallizes from ether as a solvate of colorless needles, m.p. 24–25°C. (12). The compound separates from ligroin as yellow crystals, m.p. 39°C., free of solvent. Cinnoline has a quinoline-like odor and is soluble in the usual organic solvents. The cinnolines in general have been described as colored compounds.

The ultraviolet absorption spectrum of cinnoline itself has not been described in the literature, but spectra of two closely related compounds have been determined. Ramart-Lucas and Biquard (31) reported the ultraviolet absorption spectrum of benzo[*c*]cinnoline and compared it with that of azobenzene. Evans and Wiselogle (19) have recently described the ultraviolet spectrum of pyridazine in hexane and water solutions from 2400 to 3800 Å. It appears, from infrared absorption spectrum studies on pyridazine (26a), that the ring frequencies in pyridazine are closely analogous to those in *o*-benzene-*d*₂. This indicates that there must be resonance in pyridazine of roughly the same order of magnitude as there is in benzene. One concludes that the nitrogen–nitrogen bond is neither a single nor a double bond but something intermediate. On this basis, cinnoline, which is benzo[*c*]pyridazine, would be expected to be a molecule of high resonance energy with no “fixation” of double bonds. Chemical evidence now available supports this view.

B. CHEMICAL PROPERTIES

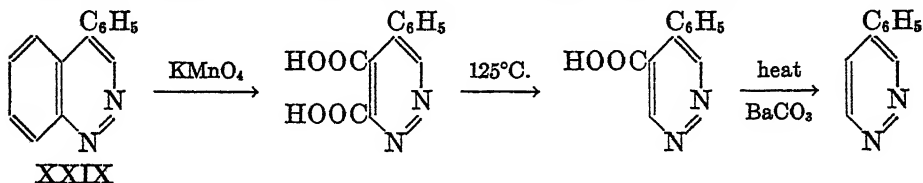
1. Salt formation

Cinnoline is a strong base and forms stable salts with hydrochloric and picric acids and an addition compound with methyl iodide (12). Most substituted cinnolines form neutral salts with acids and addition compounds with methyl iodide, ethyl iodide, and methyl sulfate (45, 56).

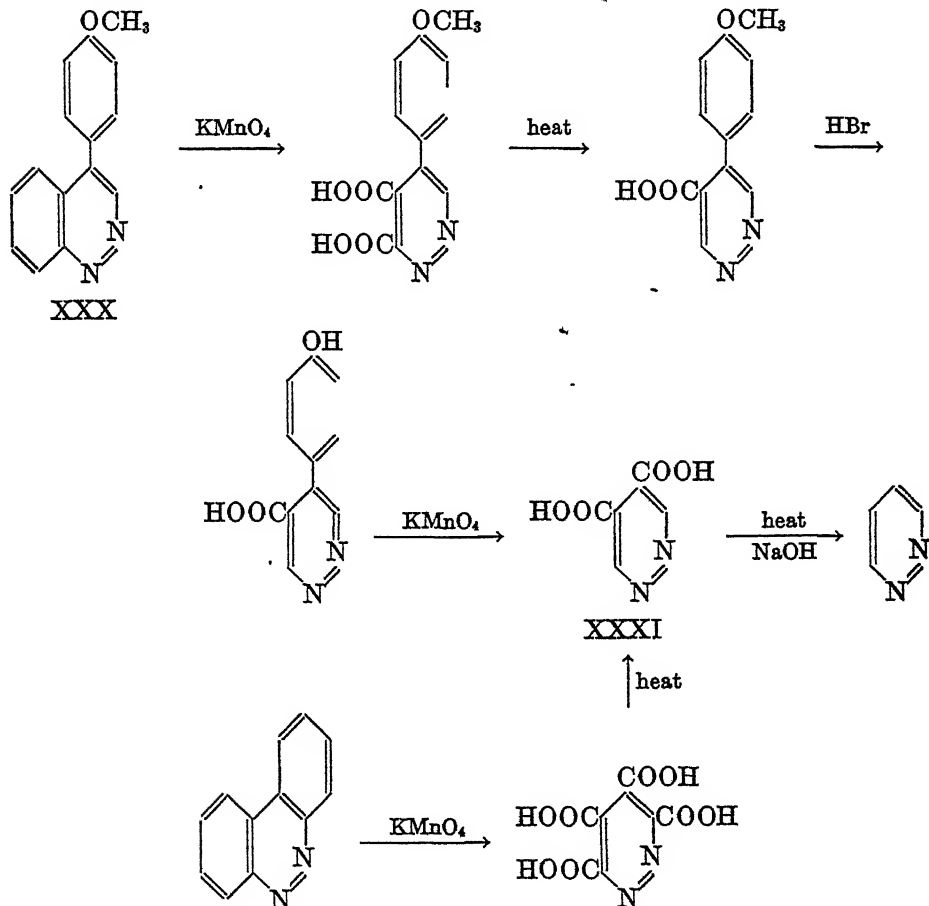
2. Oxidation

The nitrogen-containing ring in cinnoline is stable to oxidation. Potassium permanganate oxidation of 4-phenylcinnoline (XXIX) resulted in the formation

of 5-phenylpyridazine-3,4-dicarboxylic acid, which was subsequently decarboxylated in a stepwise manner to give 4-phenylpyridazine (45). Stoermer and Gaus (44) were able to show that with pyridazinedicarboxylic acids, as with pyridine-dicarboxylic acids, it is the carboxyl group nearer the nitrogen which is first

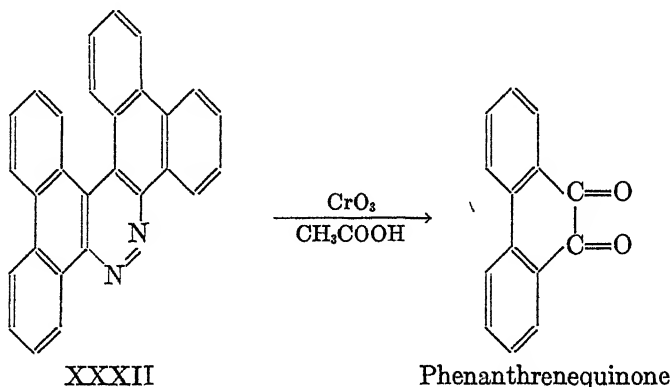


lost during decarboxylation. They oxidized 4-*p*-anisylcinnoline (XXX) and degraded the oxidation product to XXXI, which corresponded to the already known pyridazine-4,5-dicarboxylic acid.



Permanganate oxidation of benzo[*c*]cinnoline produced a pyridazinetetracarboxylic acid which was decarboxylated to the same acid, XXXI (49).

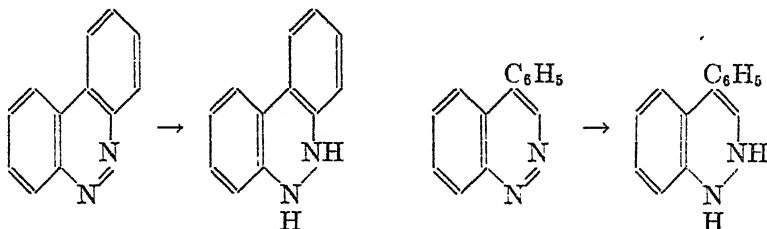
4-Phenylcinnoline (45) and benzo[*c*]cinnoline (48) resisted oxidation by chromic anhydride in acetic acid, but diphenanthro[9,10-*c*,9',10'-*e*]pyridazine (XXXII) was oxidized by chromic anhydride in acetic acid to phenanthrenequinone (39).



A few cinnolines are known to give *N*-oxides. The *N*-oxide of benzo[*c*]cinnoline was obtained by reduction of 2,2'-dinitrobiphenyl with sodium sulfide (17, 34, 51) or with the calculated amount of sodium amalgam in methanol (47), much as azoxybenzene is obtained from nitrobenzene. Excess sodium amalgam or stannous chloride in hydrochloric acid solution converted the *N*-oxide to benzo[*c*]cinnoline. The *N,N'*-dioxide of benzo[*c*]cinnoline was obtained in 25 per cent yield by reduction of 2,2'-dinitrobiphenyl with zinc and potassium hydroxide; this dioxide could be converted to the cinnoline with sodium amalgam in methanol (47).

3. Reduction

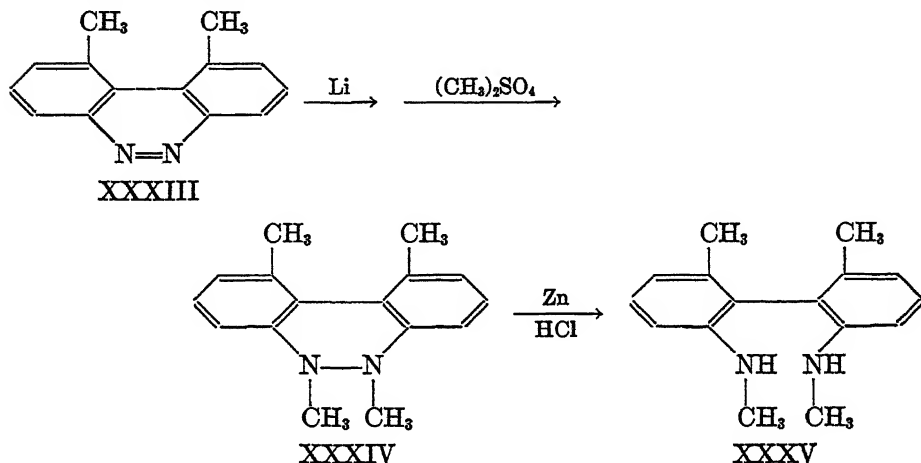
Benzo[*c*]cinnoline was reduced to 5,6-dihydrobenzo[*c*]cinnoline by means of zinc and potassium hydroxide (17), a parallel to the reduction of azobenzene to hydrazobenzene. Zinc and ethanolic ammonia brought about the reduction of 4-phenylcinnoline to 1,2-dihydro-4-phenylcinnoline (28). Iron and mineral



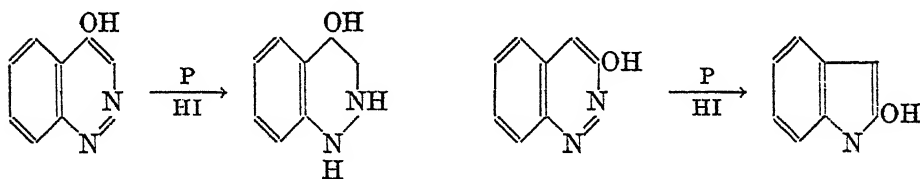
acid (12) and zinc and mineral acid (47, 55) have been used to form dihydrocinnolines, but the latter reagent when used in large excess caused the cleavage of the nitrogen-nitrogen bond. Zinc and acetic acid has likewise encouraged such cleavage in certain cinnolines (27, 39).

Cinnolines will form addition compounds with the alkali metals. Wittig

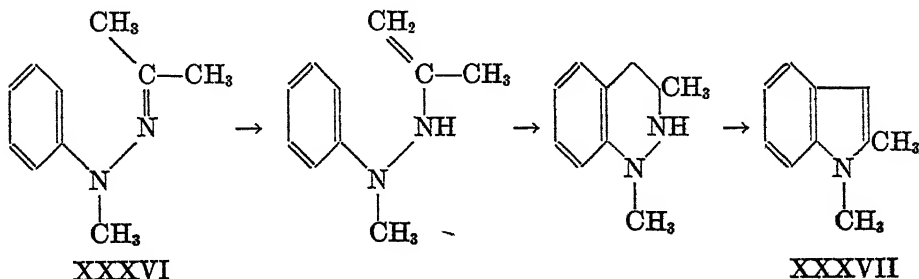
and Stiehnoth (55) found that 1,10-dimethylbenzo[*c*]cinnoline (XXXIII) formed addition products with two atoms each of either sodium, potassium, or lithium. The dilithium adduct, by treatment with methyl sulfate, formed 5,6-dihydro-1,5,6,10-tetramethylbenzo[*c*]cinnoline (XXXIV), the structure of which was proved by further reduction with zinc and hydrochloric acid to the substituted biphenyl, XXXV.



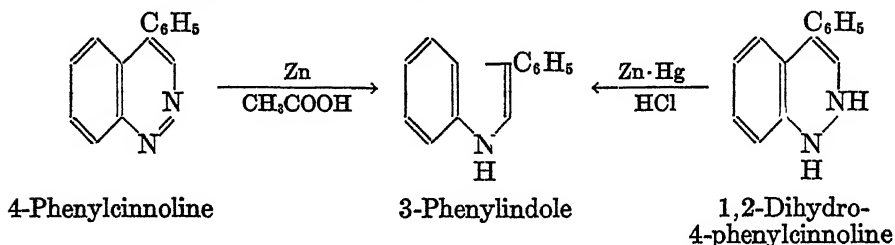
Reduction of two hydroxycinnolines with phosphorus and hydriodic acid was carried out by Neber (28), who found that 4-hydroxycinnoline was converted to the tetrahydro derivative and 3-hydroxycinnoline underwent rearrangement to oxindole.



He suggested at one time that tetrahydrocinnolines might be intermediates in the Fischer indole synthesis, as represented by XXXVI to XXXVII, but later carried out reduction studies in the cinnoline series which showed this view to



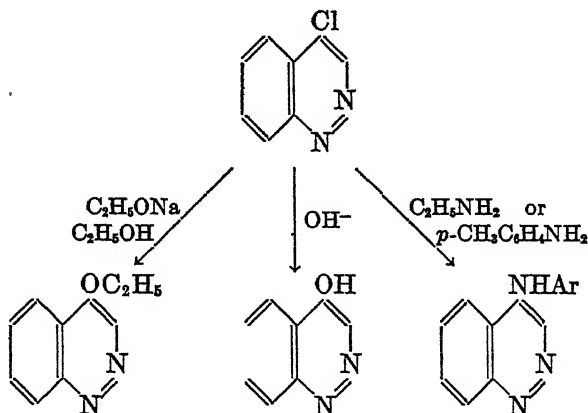
be untenable. 4-Phenylcinnoline and 1,2-dihydro-4-phenylcinnoline were converted to 3-phenylindole by treatment with zinc in acid solution.



If the reaction proceeded through the tetrahydrocinnoline, then 1,2,3,4-tetrahydro-4-phenylcinnoline (prepared from the dihydrocinnoline by catalytic hydrogenation over platinum oxide catalyst) should have given the same product, 3-phenylindole. However, 1,2,3,4-tetrahydro-4-phenylcinnoline remained unchanged when subjected to the same conditions. 1,2-Dihydro-4-phenylcinnoline, when heated at 100°C., underwent disproportionation to 4-phenylcinnoline and 1,2,3,4-tetrahydro-4-phenylcinnoline.

4. Replacement reactions

No nuclear substitution reaction has been carried out on the cinnoline molecule. Replacement reactions studied have been limited to those of 4-chlorocinnoline (11), prepared in 90 per cent yield from 4-hydroxycinnoline by phosphorus oxychloride and phosphorus pentachloride. The 4-chloro group was replaced readily with an ethoxyl group, an aromatic amino group, or again with a hydroxyl group.



The high reactivity of chlorine in the 4-position is similar to that of the chlorine in 4-chloroquinoline.

V. SUMMARY

The methods of synthesis and the physical and chemical properties of cinnolines have been reviewed.

Cinnolines have been made from diazotized *o*-aminophenylpropionic acids, *o*-aminophenylethylenes, and *o*-aminoacetophenones. The nature of the substituent groups in the *o*-aminophenylethylenes has a delimiting effect upon the applicability of this ring-closure method.

A special method for the preparation of polynuclear cinnoline derivatives depends upon the reaction of hydrazine with 1,4-diketones. Certain benzo[*c*]-cinnolines have been formed from diazo compounds or substituted 2,2'-dinitrobiphenyls. Two cinnoline derivatives have been made by means of a Diels-Alder condensation.

The cinnolines are basic compounds which readily undergo reduction to dihydrocinnolines. The nitrogen-containing ring of cinnoline is stable to oxidation.

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COLLOID CHEMISTRY OF CLAYS

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CONTENTS

I. Introduction—the colloidal state of matter.....	287
II. Chemical composition, structure, and shape of clay minerals.....	290
A. X-ray diffraction studies.....	292
B. Electron-microscope studies.....	292
III. The clay mineral particle—a colloidal micelle.....	295
IV. Colloidal phenomena and properties of clays.....	301
A. Ion exchange.....	302
B. Plasticity.....	305
C. Dispersion.....	308
D. Dilatancy.....	308
E. Thixotropy.....	309
F. Rheopexy.....	310
G. Stream double refraction.....	312
V. Applied colloid chemistry of clays.....	314
A. Ceramics.....	314
B. Agriculture.....	314
C. Soil engineering.....	315
D. Drilling muds.....	315
E. Films and plastics.....	315
F. Thixotropic rubber compounds.....	317
G. Thixotropic adhesives.....	317
H. Water softening.....	319
VI. References.....	319

Colloids are not to be considered as a separate group of substances, but rather as matter in a state characterized by an overwhelming development of surface over total volume. This condition causes matter to exhibit reactivity which is not predictable on the basis of chemical composition alone. This is exemplified by a detailed discussion of the results of chemical analysis of the most important clay minerals, their shape and structure as revealed by x-ray diffraction and electron microscopy, and their colloidal properties. The combination of these findings is used to describe the clay particle as a colloidal micelle. This concept, which has proved to be of greatest value in explaining many phenomena typical of clay minerals, is applied in discussing ion-exchange reactions, plasticity, thixotropy, dilatancy, rheopexy, dispersion, and stream double refraction. The industrial application of these colloidal phenomena is exemplified by a discussion of the use and properties of clay minerals in ceramics, agriculture, soil engineering, oil well drilling, films and plastics, thixotropic pastes, and water softening.

I. INTRODUCTION—THE COLLOIDAL STATE OF MATTER

Many are still under the impression that the term "colloid" refers only to gelatinous substances or to tiny particles being moved around in the medium in which they are dispersed by the Brownian molecular motion. The term "colloid"

was coined by Thomas Graham in 1861. It is derived from the Greek words *κόλλα* (kolla) meaning *glue*, and *εἶδω* (eido) or *εἶδομαι* (eidomei) meaning *like*. Many even still believe that the term "colloid" defines a special group of chemicals, like the words *metal*, *wood*, or *wax*, and that colloid chemistry, therefore, is comparable in its scope to metallurgy or mineralogy. Therefore, it seems necessary to point out at the very outset of a paper dealing with the colloid chemistry of clays that this branch of science is not limited in its scope (like inorganic or organic chemistry) to well-defined groups of chemical compounds, but is the science devoted to the exceptional reactivity and phenomena exhibited by matter if present in the dimensional range from 1 to 500 μ .

The fact that a substance having one, two, or all three possible dimensions lying within this range will exhibit properties which cannot be predicted or explained by simple analysis or by the reasoning of classical chemistry calls for an explanation. If we take, for example, a cube with 1 mm. edge length, a particle clearly visible in a good microscope, it will have a volume of 1 mm.³, a total surface of 6 mm.², eight corners, and twelve edges. If we now subdivide it into cubes of 500 μ edge length, thus producing particles of a size which has been set as the upper limit of the colloidal range, we shall have produced eight billion cubes. Their combined volume will still be 1 mm.³, but the sum of the now formed surfaces will amount to the staggering figure of 12,000 mm.² and there will be 64 billion corners and 96 billion edges.

As we further decrease the dimensions of the formed particles, the ratio of volume to exposed surface will become even more pronounced, until we have reached the dimensional range of the individual atoms or ions of which the matter under consideration is composed. Then any differentiation between volume and surface becomes meaningless, at least as far as the chemical compound is concerned. We are then dealing with a gas or a dispersion of dissociated ions, a true solution.

If we look at a model of a bromargyrite (silver bromide) crystal, for example (figure 1), we immediately realize why so much importance must be attached to the surface-to-volume ratio of matter if present in the colloidal state. It is evident that the silver ion (1) should be surrounded by six bromide ions. Only four are visible; the fifth is located behind the silver ion inside of the cube, and the sixth is missing altogether. The corner ion (3) lacks three bromide ions for electrical neutrality. This deficiency, so pronounced in crystalline matter present in a colloidal state of subdivision, as the clay minerals are, explains why they exhibit a reactivity and certain properties which could not be explained on the basis of their chemical composition alone. To achieve neutrality, these ions tend to adsorb positive or negative charges, respectively, from the medium which surrounds them. In this way the so-called diffuse double layer is built up. This layer is composed of those ions which are firmly adsorbed to the surface of the particle and the counter ions which surround the particle at a distance which depends on the strength of their electrostatic attraction and their solvation. The particle plus its diffuse double layer is called a colloidal micelle. Only if the concentration of counter ions is high, owing to an excess of electrolyte

present, or if they are multivalent, will the effective charge carried by the colloidal micelle be neutralized. Nature always tends to produce matter exhibiting least free energy. Therefore, in the above case there will always be the tendency to form the least soluble compound, and should the reaction lead to the formation of crystalline matter, those ions which fit into the crystal lattice will be preferably adsorbed, in accordance with the Fajans-Hahn rule (38).

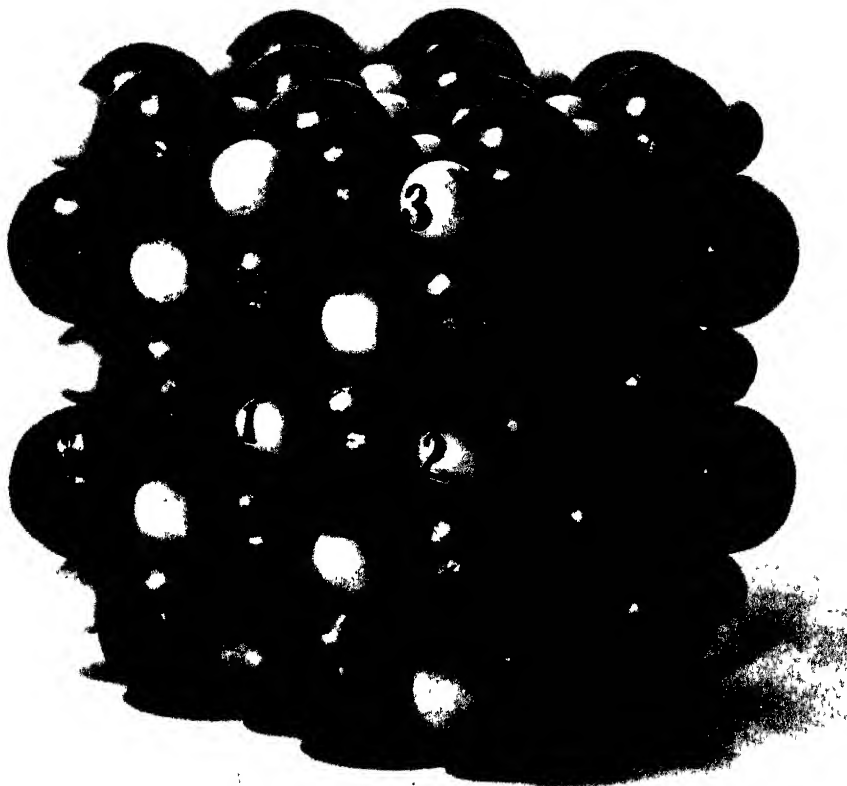


FIG. 1. Close-packed ionic lattice model of a bromargyrite crystal (39). (A detailed description of how to build such close-packed crystal models simply and quickly is given in reference 40.)

If we, therefore, let silver nitrate react with potassium bromide and the concentrations used are not in exactly stoichiometric proportions, then the electric sign imposed on the formed colloidal micelle will depend on the type of ions present in excess. If the reaction is carried out with an excess of silver nitrate, then the residual silver ions will be adsorbed by the unsaturated secondary valencies of the bromide ions located in the surface of the created crystal and positively charged particles will result. If the reaction is carried out with an excess of potassium bromide, the bromide ions will be adsorbed on the silver

ions of the crystal lattice, resulting in negatively charged particles, with potassium ions forming the counter ions (figure 2). The introduction of the concept of a diffuse double layer by Gouy (27) and Freundlich (16) overcame the shortcomings of the double-layer theory originally postulated by Helmholtz (53), which assumed that the surface charge of the colloidal particle is compensated by a counter-ion layer located at monomolecular distance in the dispersion medium (figure 3). The new concept has not only offered a better understanding of the differences between the thermodynamic or galvanic potential (38) and the electrokinetic potential, but has also offered a logical explanation for the fact that the former is independent of ion concentration and solvation, whereas the latter is very much influenced thereby (38).

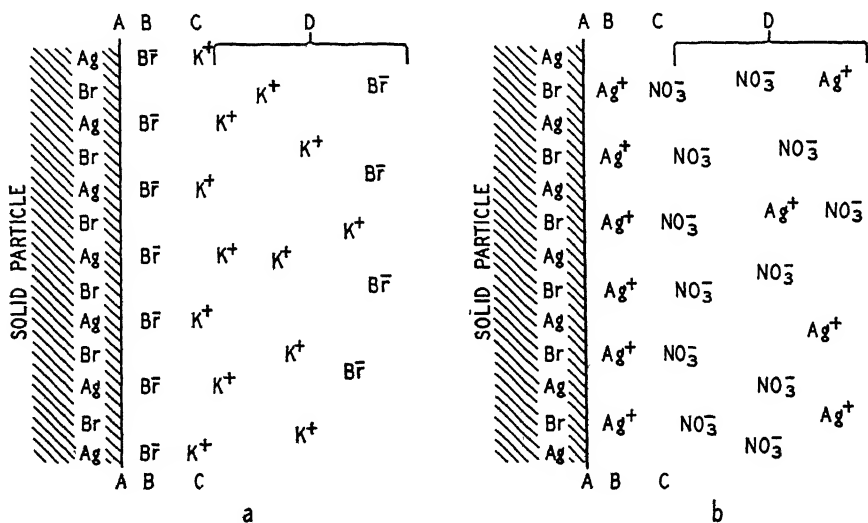


FIG. 2. Arrangement of ions in diffuse double layer surrounding a silver bromide particle. a, negatively charged particle; b, positively charged particle. A, actual surface of particle; B, rigid ionic layer firmly attached to particle; D, diffuse ionic atmosphere in movable part of liquid; C, imaginary boundary between attached non-movable and movable liquid layers (38).

If the counter ions are easily solvated, like sodium, the colloidal micelle will, when placed in water, build up a diffuse electric double layer due to the surface dissociation of the counter ions. It is this phenomenon which is most responsible for several of the unique properties exhibited by colloidal clays, and it is this condition of the clay minerals which makes a more fundamental knowledge of their colloid chemistry so important to science and industry.

II. CHEMICAL COMPOSITION, STRUCTURE, AND SHAPE OF CLAY MINERALS

If the most important clay minerals are subjected to quantitative chemical analysis, one finds, as shown in table 1, that this knowledge alone does not suffice to explain either the differences in properties some of the clays exhibit,

although their composition, as found by analysis, is the same, or why some have similar properties, although they differ in chemical composition. Some of the

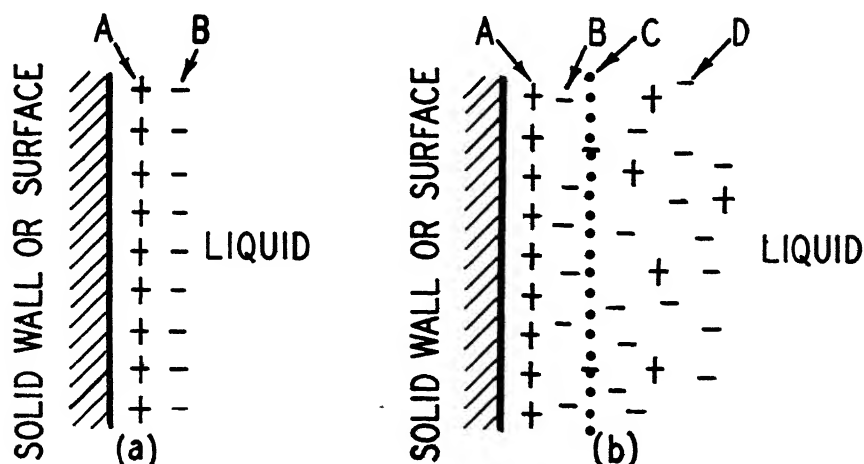


FIG. 3. Double layers. (a) Helmholtz; (b) Gouy-Freundlich diffuse double layer. A, charges firmly adsorbed to particle; Ba, charges in liquid layer, Bb, ions in liquid layer firmly attached to particle; C, thickness of attached layer; D, diffuse ions in movable part of liquid (38).

TABLE 1

MINERAL	CHEMICAL COMPOSITION*	TYPE OF CRYSTAL LATTICE
Kaolinite	$\text{Al}_4(\text{Si}_4\text{O}_{10})(\text{OH})_8$	Kaolinite
Dickite	$\text{Al}_4(\text{Si}_4\text{O}_{10})(\text{OH})_8$	
Nacrite .	$\text{Al}_4(\text{Si}_4\text{O}_{10})(\text{OH})_8$	
Halloysite. . .	$\text{Al}_4(\text{Si}_4\text{O}_8)(\text{OH})_{16}$	
Metahalloysite. . .	$\text{Al}_4(\text{Si}_4\text{O}_{10})(\text{OH})_8$	
Attapulgite (2) .	$\text{Mg}_5[\text{Al}](\text{Si}_8\text{O}_{20})(\text{OH})_4(\text{OH})_2 \cdot 4\text{H}_2\text{O}$	Montmorillonite
Pyrophyllite...	$\text{Al}_4(\text{Si}_8\text{O}_{20})(\text{OH})_4$	
Talc .	$\text{Mg}_3(\text{Si}_2\text{O}_5)(\text{OH})_4$	
Montmorillonite	$\text{Al}_4[\text{Mg}](\text{Si}_8\text{O}_{20})(\text{OH})_4 \cdot x\text{H}_2\text{O}$	
Nontronite .	$\text{Fe}_4[\text{Mg}](\text{Si}_8\text{O}_{20})(\text{OH})_4 \cdot x\text{H}_2\text{O}$	
Beidellite ...	$\text{Al}_4[\text{Mg}](\text{Si}_8[\text{Al}]\text{O}_{20})(\text{OH})_4 \cdot x\text{H}_2\text{O}$	
Saponite ...	$\text{Mg}_5(\text{Si}_8[\text{Mg}]\text{O}_{20})(\text{OH})_4 \cdot x\text{H}_2\text{O}$	
Illite (1)...	$\text{K}_y \cdot \text{Al}_4(\text{Fe}_4 \cdot \text{Mg}_4 \cdot \text{Mg}_6)(\text{Si}_8 - y \cdot \text{Al}_y)\text{O}_{20}^\dagger$	Illite
Muscovite	$\text{K}_2 \cdot \text{Al}_4(\text{Al}_2\text{Si}_6\text{O}_{20})(\text{OH})_4$	Mica

* Symbols in [] indicate that they may substitute for the symbol written to the left of the bracket.

† According to Grim (28), y varies from 1 to 1.5.

reactions, like gelation, led to the assumption that clays represent matter in the amorphous state. The introduction of two new research tools, the x-ray diffrac-

tion technique and the electron microscope, demonstrated that we are dealing with matter in crystalline form. A careful survey of these facts, coupled with colloid-chemical deductions, soon led to the conclusion that the great variations in properties could be accounted for only by assuming that the clay minerals are composed of comparatively simple building units and that the differences are primarily due to the manner in which these units are put together.

A. X-ray diffraction

Just over twenty years ago Hadding (32) and Rinne (86) proved by x-ray diffraction that clays were not composed of matter in the amorphous state, as had so far been assumed, but were crystalline. Since then our knowledge of the structure of clays has been greatly increased by the application of x-ray diffraction analysis (10, 28, 31, 43, 52, 54, 55, 57, 58, 62, 63, 68, 69, 81). This development, coupled with a more systematic study of the properties exhibited by colloidal clays, has not only led to the establishment of what we today term the clay mineral group, but has also offered a better understanding of some of the phenomena which are so characteristic of colloidal clays and which could not be explained on the basis of purely physicochemical reasoning.




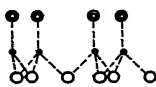
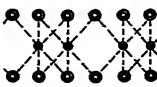
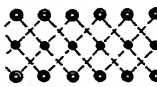
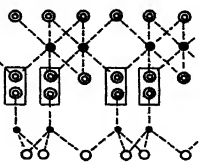
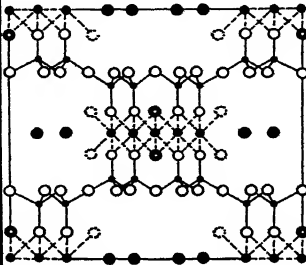
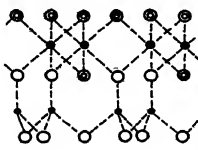
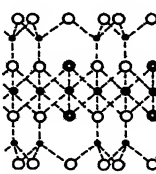
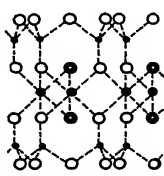
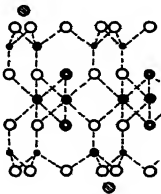
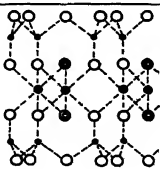
One might compare the structure of the clay minerals with houses built of bricks. One can build houses of different shapes and sizes with the same bricks and mortar. Table 2, based on x-ray diffraction studies, offers the proof for this point of view (39). Columns 1, 2, 3, and 4 give a schematic picture of the bricks used. The mortar used in the building of the clay minerals is the unsaturation of their ultimate building units. For example, a silicon atom will share four electrons with a neighboring atom ($\text{Si} = +4$). An oxygen atom needs two electrons for saturation ($\text{O} = -2$). Therefore, the silicon-oxygen tetrahedron (SiO_4) is not saturated. If several of these tetrahedra combine by sharing oxygen atoms, a chainlike structure results (figure 4). In such a chain, two of the oxygen atoms belonging to every silicon atom remain unsaturated. This deficiency can be compensated for by adsorbing, for example, sodium ions. This will result in a fibrillar aggregate known as sodium silicate or water glass. The fibrous structure and the high degree of hydration of the sodium ion explain the high viscosity exhibited by solutions of water glass. A careful study of table 2 will reveal that all the clay minerals show points of unsaturation at their edges (figure 5) and some, like substituted montmorillonite or mica, even within the crystal lattice. This concept, therefore, offers a simple explanation of many of the properties which are typical of the clay minerals and for which no really satisfactory answer was available on the basis of the chemical composition alone.

B. Electron-microscope studies

Up until only a few years ago our concepts of the shape and the actual dimensions of colloidal clay particles were largely based on deductions derived from combining the findings of chemical analysis, x-ray diffraction patterns, and colloidal phenomena exhibited by the various clay minerals. The development

TABLE 2

Structural data of the most important clay minerals and their building units

<div>1</div>  <div>1 O 1 Si 3 O</div>	<div>2</div>  <div>3 OH 1 Al 3 OH</div>	<div>3</div>  <div>3 OH 1 Mg 3 OH</div>
<div>4</div>  <div>4 OH 4 Si 6 O</div>	<div>5</div>  <div>6 OH 4 Al 6 OH</div>	<div>6</div>  <div>6 OH 6 Mg 6 OH</div>
<div>7</div>  <div>6 OH 4 Al 6 OH 4 OH 4 Si 6 O</div>	<div>8</div>  <div>6 Mg OR Al + 4 H₂O 2 OH + 4 O + 2 OH₂ 4 Si 13 O 4 Si 2 OH₂ + 4 O + 7 OH 4 H₂O + 5 Mg OR Al 2 OH₂ + 4 O + 1 OH 4 Si 13 O 4 Si 2 OH + 4 O + 2 OH₂ 6 Mg OR Al + 4 H₂O</div>	
<div>9</div>  <div>6 OH 4 Al 4 O + 2 OH 4 Si 6 O</div>	<div>10</div>  <div>6 O 4 Si 4 O + 2 OH 6 Mg 4 O + 2 OH 4 Si 6 O</div>	
<div>11</div>  <div>6 O 4 Si 4 O + 2 OH 4 Fe⁺⁺⁺ 4 O + 2 OH 4 Si 6 O</div>	<div>12</div>  <div>1 K 6 O 3 Si + 1 Al 4 O + 2 OH 4 Al 4 O + 2 OH 3 Si + 1 Al 6 O 1 K</div>	
<div>13</div>  <div>6 O 4 Si 4 O + 2 OH 3 Al + 1 Mg 4 O + 2 OH 4 Si 6 O</div>	<div>Legend:</div> <ul style="list-style-type: none"> • - Si ● - Mg, Al, Fe⁺⁺⁺ ○ - O ● - OH ● - K ● - H₂O ○ - OH₂ 	

The schematic drawing in the first column of each section represents the composition of the unit cell of the respective building unit or complete crystal lattice. All atoms have been projected into one plane. The second columns give the number and type of atoms or groups for every lattice plane. 1 = silicon tetrahedron; 2 = aluminum octahedron; 3 = magnesium octahedron; 4 = hydrated silica; 5 = gibbsite; 6 = brucite; 7 = halloysite; 8 = attapulgite; 9 = kaolinite; 10 = talc; 11 = nontronite; 12 = mica (illite); 13 = montmorillonite (substituted).

This table is a rearrangement of illustrations previously published by Grim (28) and

of the electron microscope has now put a new research tool in our hands which enables us actually to see, study, and measure particles whose sizes fall within the smallest range of colloidal dimensions (84).

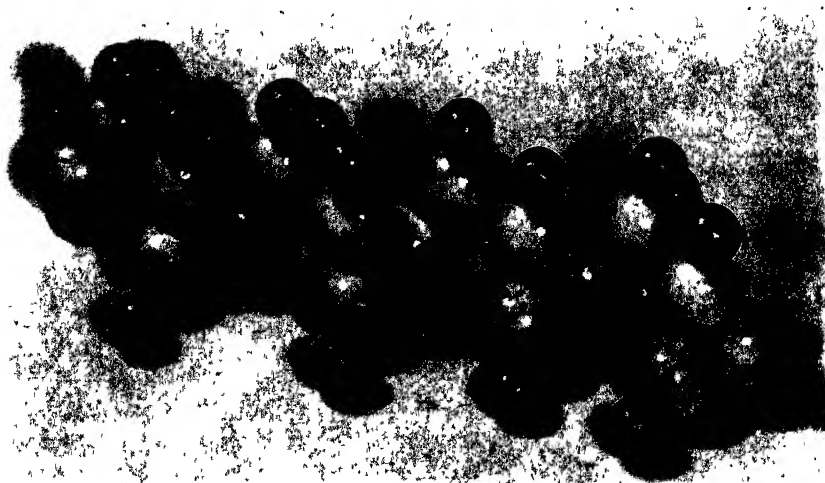


FIG. 4. Model of a sodium silicate chain (theoretical); white balls represent oxygen atoms; black balls, sodium atoms. The silicon atoms are not visible, because they are located in the cavity formed by the four oxygen atoms in tetrahedral position. It is clearly evident that two sodium atoms are needed for the saturation of every tetrahedron (40).

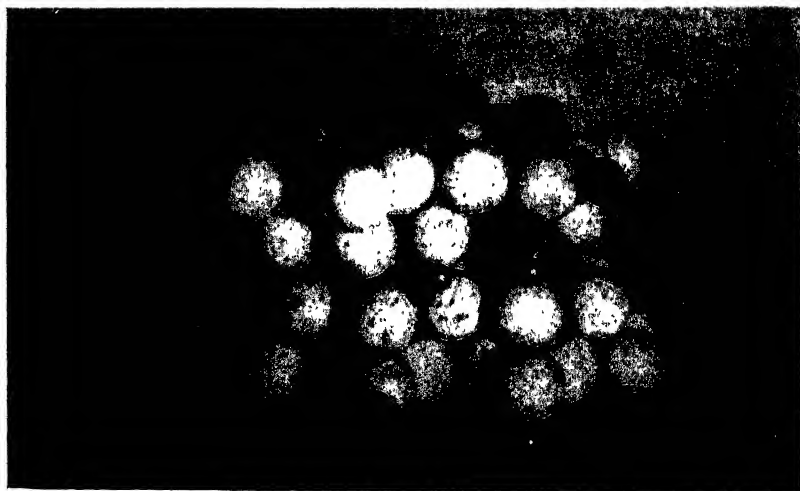


FIG. 5. Model of a single montmorillonite crystallite. The white balls represent oxygen; the black, hydroxyl groups; the small metallic balls, aluminum; the gray balls on the edges, adsorbed cations, for example, sodium (40).

The first to use this new research tool for the study of clays were the Germans, who thereby proved that the particles of bentonite and kaolin actually were of platy shape (1, 2, 11, 12, 13, 14, 79, 84). More detailed studies were carried out

shortly thereafter in this country, and were reported in the excellent summarizing publications of Marshall, Shaw, Humbert (73, 89, 59, 90), and collaborators. Figure 6 shows that the particles of kaolinite are thin plates. Figure 7 shows dickite crystals. Figure 8 represents an electron microphotograph of Wyoming bentonite, which clearly shows that its crystallinity is not as well developed as that of the previously mentioned clays. The clay mineral attapulgite consists of fibrous particles, as shown in figure 9, and the same is the case with the clay mineral halloysite (84, 90).

These findings offer for the first time an explanation for the difference in colloidal properties of the last-mentioned two clay minerals and those exhibited by kaolinite and talc, respectively. The recently made assumption that halloysite and attapulgite should be considered as intermediary products in the



FIG. 6. Electron microphotograph of kaolinite (89)

formation of kaolinite and talc from hydrated silica and hydrated aluminum or magnesium or mixtures of the latter two therefore seems very plausible (28, 39).

III. THE CLAY PARTICLE—A COLLOIDAL MICELLE

The electrical forces associated with a colloidal particle in an electrolytic medium arise from two sources. One set arises from the ions which are rigidly bound to the surface of the particle, and the other is due to the ions of opposite sign which concentrate around the particle in an effort to neutralize its charge. Inasmuch as the particle still carries a net charge, the number of counter ions must be insufficient for neutralization. Since some ions of opposite sign are randomly floating in the dispersion medium, it will carry a charge opposite to that of the colloidal micelle. Figure 10 gives an idea of the relative magnitude

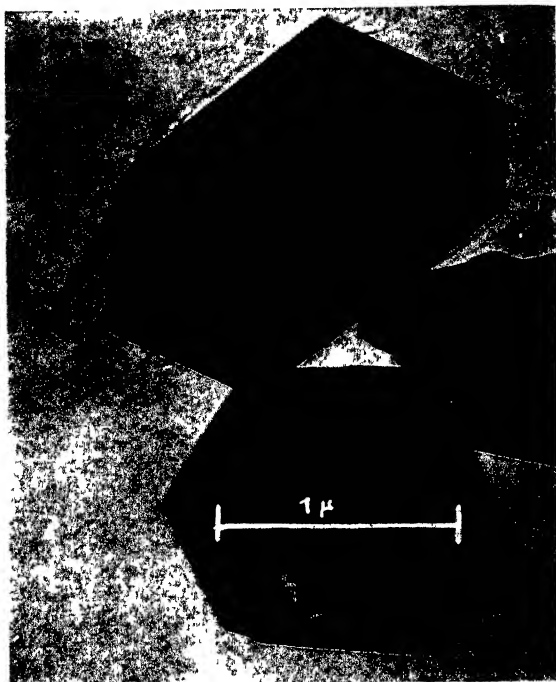


FIG. 7. Electron microphotograph of dickite crystals (59)

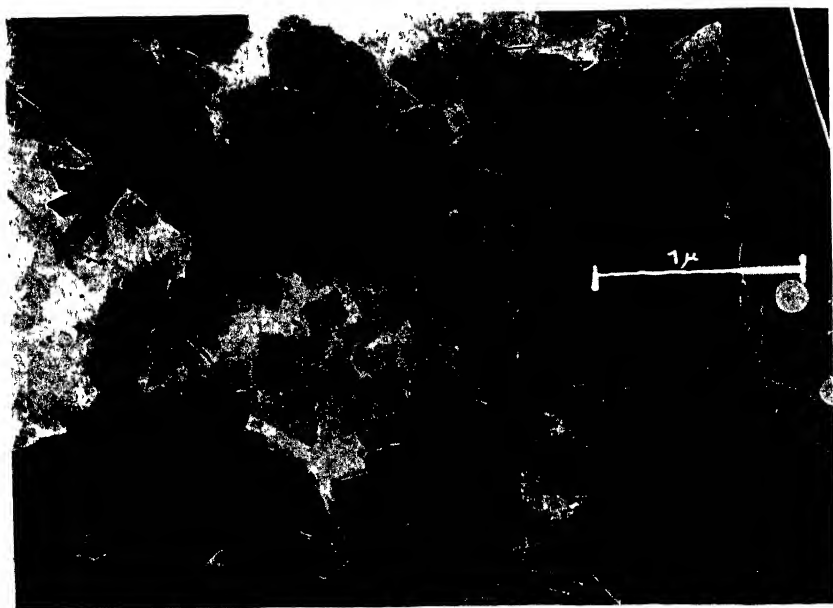


FIG. 8. Electron microphotograph of bentonite (89)

of the electrical forces associated with a colloidal micelle. Line A represents the rate of decline of the electrical forces due to the basic charge of the particle with distance from the surface of the actual particle. Line R shows the decline of the electrical forces associated with the diffuse double layer of net opposite sign. The vertical axis intersecting A and R is the center from which all the ions of the diffuse layer can be assumed to act. If the particle, as is the case with particles of colloidal clays, carries a net negative charge, then curve A would represent the forces of attraction for positive ions in the dispersion medium and curve R the forces of repulsion due to the outer layer of the colloidal micelle toward other similar particles dispersed in the system. We can see that the



FIG. 9. Electron microphotograph of attapulgite (73)

repulsion forces have the upper hand beyond the diffuse double layer, thus preventing ions of sign opposite to that carried by the particle from coming into close proximity and causing neutralization of the charge and also preventing a close approach by other colloidal micelles of identical composition.

Owing to the adsorption of ions from the dispersion medium by the colloidal particle or to the dissociation of ions from its surface into the dispersion medium, as is typical for clays of the bentonite group, the dispersion medium will possess an excess of ions of a charge opposite to that of the particles. This is evidenced by the phenomenon known as the electroviscous effect. As Smoluchowski has shown, the electroviscous effect is due to a drag caused by the dispersion medium carrying the counter charges moving towards one pole and the dispersed particles moving to the other pole in an electric field (91). This electric field, if of suffi-

cient strength, can cause condensation and orientation of the colloidal micelles (26, 46). This condition will exist until the attraction forces resulting from the charge on the particles and the ions in the dispersion medium are in balance with the forces of repulsion between the ions in the adsorbed diffuse layer and those freely moving in the dispersion medium. Thus, a thixotropic gel (18) will form, but it will immediately revert to a sol if the free movable ions are

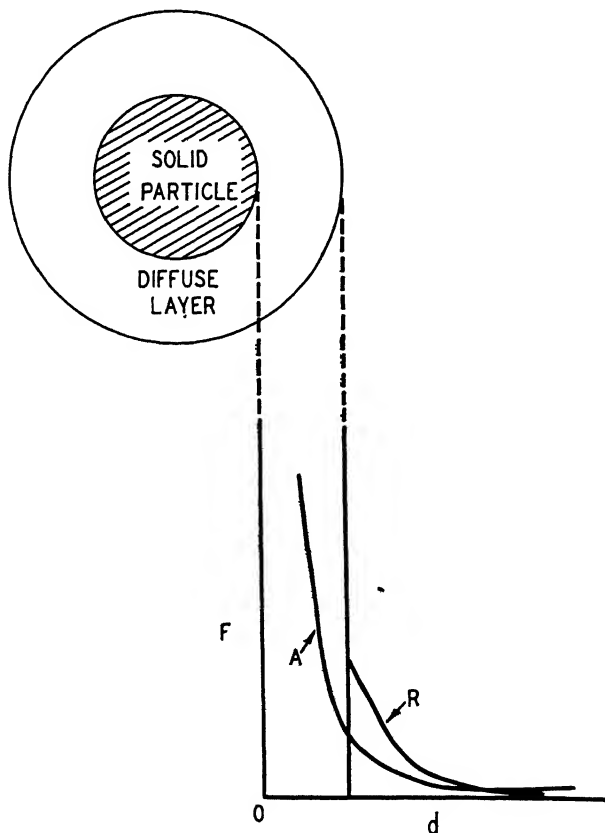


FIG. 10. A schematic picture of the forces associated with the charge of a colloidal particle, the diffuse layer, and any given charge possessing the same sign as the particle, as a function of the distances separating them (46).

redistributed, for example by agitation. If the concentration, valency, or hydration of the counter ions is such that the magnitude of the net charge of the colloidal micelle or the thickness of the diffuse double layer is so reduced that van der Waals attraction forces come into play, coagulation will result, as shown in figure 11.

This concept of the colloidal micelle and of the electric forces connected with it has finally enabled us to offer a simple explanation for many of the properties which are so characteristic of clays and which up to not so long ago baffled the

chemist who could only rely on the results of chemical analysis of a substance. However, before discussing some of them (like plasticity, thixotropy, and rheopexy) in detail, it seems advisable first to lay the foundation for the clay particle

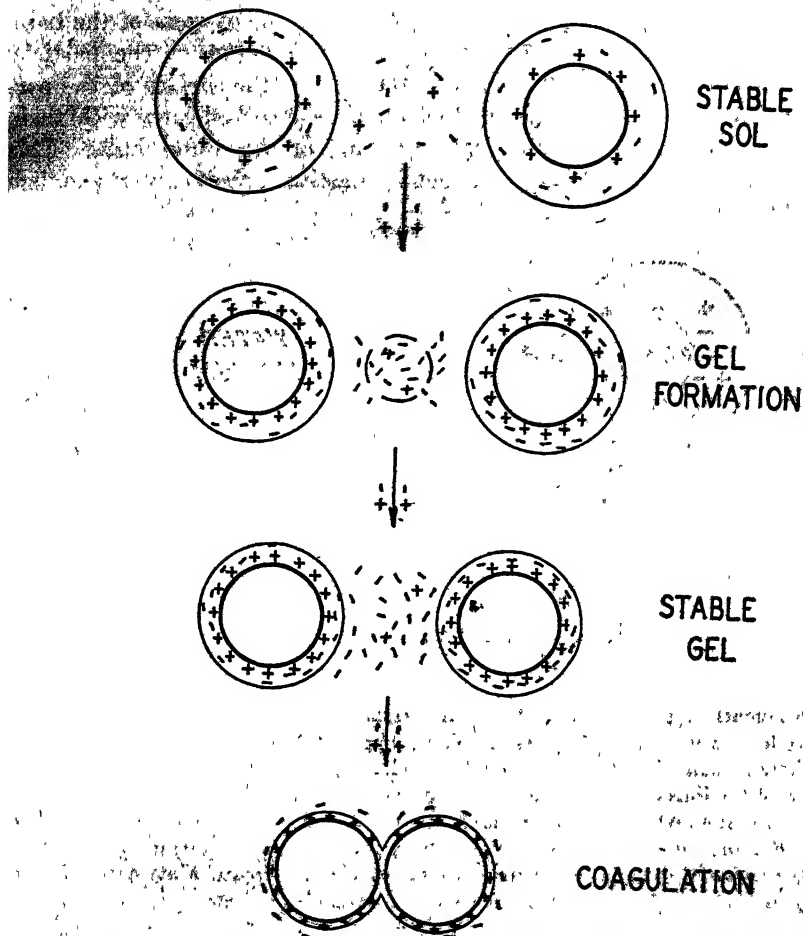


FIG. 11. Coagulation of a colloid by electrolyte. Schematic, simplified picture of the mechanism of gelation and coagulation, showing the formation of ionic fields in the dispersion medium due to preferential adsorption of ions on the surface of the particles. The first step in gel formation corresponds to a thixotropic condition (46).

as a colloidal micelle. Let us, for example, look at a kaolinite particle dispersed in pure water.

Kaolinite, according to its crystallographic classification, is a monoclinic system and therefore displays good cleavage. In the formation of fragments, the fracture occurs along the basal cleavage plane and normal to it, forming thin hexagonal platy particles, but fracture along the cleavage plane does not cause the rupture of primary valence bonds. In a fracture parallel to the *c*-axis of

the crystal unit, however, it becomes necessary to break the bonds between Si and O, Al and OH, or O. (For a schematic drawing of a kaolinite crystal lattice, see table 2, 9.) These broken bonds serve as the basis for the preferential adsorption of hydroxyl ions. The fact, moreover, should not be overlooked that these hydroxyl ions may be bonded by dipolar bonds to the oxygens of the basal silica sheet. Therefore, in order to set up a negative charge on the particle, the location and mechanism of attachment of anions on the surface of the particle are irrelevant. The hydroxyl ions that are thus adsorbed carry with them water molecules (hydration) which make up part of the water hulls. This particle and the cations which swarm about it constitute the Gouy-Freundlich diffuse double layer (figure 12).

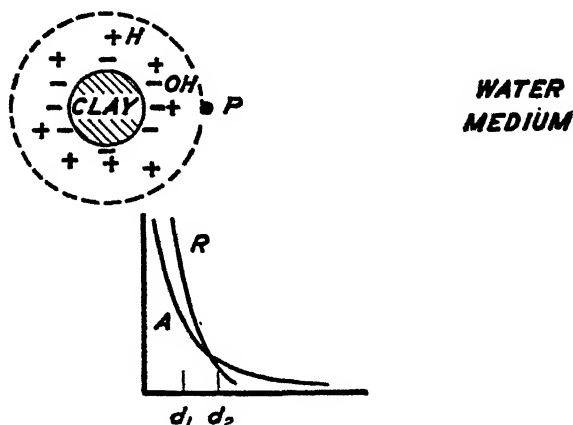


FIG. 12. Schematic representation of the arrangement of the ions associated with a kaolinite micelle in a watery medium (47). The broken circle represents the edge of the diffuse lyosphere associated with the colloidal particle. d_1 represents the average distance from which all of the ions of the diffuse double layer act. At the distance d_2 , P represents the point in the system where the charge associated with the colloidal micelle approaches zero. Curve R represents the decrease of repulsive forces due to the counter ions associated with the diffuse double layer. Curve A represents the decrease of attraction forces due to the hydroxyl ions which give the negative charge to the particle.

If a small trace of an electrolyte (sodium hydroxide) is added to this system, profound changes in the forces connected with the micelle will occur as a result of the preferential ion adsorption on the particle and of the rearrangement of counter charges. Figure 13 shows such an arrangement of the ions of the system and of its attraction-repulsion forces as a function of distance. A considerable repulsion force exists at the edge of the lyosphere, that is, the charge density on the particle and its potential is high, and the entire system is at maximum stability from a colloidal viewpoint because of the presence of large repulsive forces between particles. A kaolinite slip in this state is said to be deflocculated or dispersed (47, 60).

If we now look at the structure of a clay mineral of the montmorillonite group, exhibiting, as is most common, a substituted lattice (table 2, 13) and not the

ideal structure, we find that these substitutions result in establishing net residual negative charges in certain sections of the lattice, which, in turn, cause cation attraction. Sodium is the most common cation found in naturally occurring American bentonites. The crystal units of montmorillonite are only loosely held together by weak O—O bonds. The sodium ions are primarily adsorbed on the surfaces of the silica sheets and to a negligible extent only on the fractures along the *c*-axis. When hydration occurs, these ions pry the particles apart and form double layers of appreciable thickness. The clay exhibits a high degree of swelling. This assumption has been substantiated by x-ray diffraction patterns, revealing a so-called “expanding lattice” along the *c*-axis of the mineral (39).

In the formation of bentonite particles fractures parallel to the *c*-axis, similar to those occurring in the kaolinite crystal, seldom take place. On these surfaces

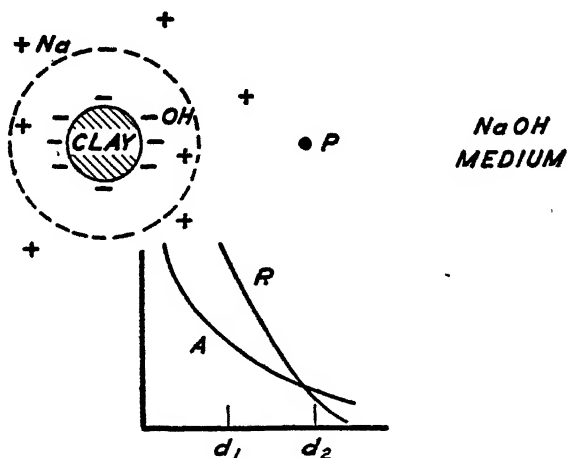


FIG. 13. Schematic representation of the arrangement of the ions associated with a kaolinite micelle in an alkaline medium (47.) (For an explanation of the lettering, see figure 12.)

a diffuse double layer, identical with that obtained with kaolinite, can theoretically be formed, that is, first a layer of preferentially adsorbed hydroxyl ions and then the counter ions in a diffuse arrangement.

With this as a basis, we can now discuss the most important properties of the clay minerals far more intelligently than has so far been possible and thereby demonstrate the importance colloid chemistry has in any field of science and technology in which clays are encountered or used.

IV. COLLOIDAL PHENOMENA AND PROPERTIES OF CLAYS

On the preceding pages the attempt was made to familiarize the reader with the real meaning of the term “colloidal state of matter” and to offer as simple as possible an explanation why matter, if present in that state, will exhibit properties quite unique to that condition. The main purpose of this was to explain

why a clay particle—not only because of its size and shape, but also because of its chemical composition—must, when dispersed in water or a solution of electrolytes, be regarded as a colloidal micelle. In the following pages proof for the importance of colloid chemistry in explaining some of the phenomena and properties which are so characteristic of clays will be offered.

A. Ion exchange¹

The most important and interesting phenomenon exhibited by those clay minerals which will in contact with water form a colloidal micelle is the ion-exchange reaction. This reaction was originally recognized by Thompson in his investigation on the properties of soils (95); the first systematic work upon it was done by Way (99).

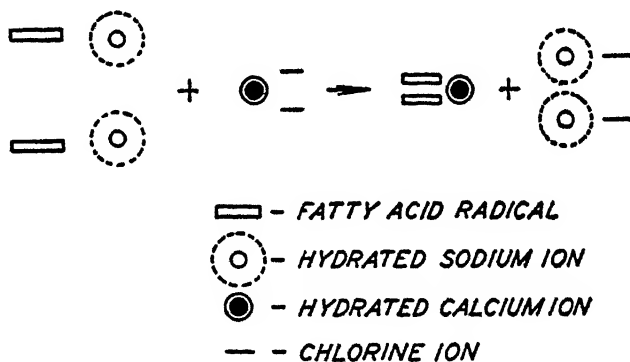


FIG. 14. Schematic representation of the formation of a less dissociated and less soluble soap by cation exchange.

As has been shown, the ultimate clay crystal carries a net negative charge. This charge is either the result of anion adsorption onto its surface, or it can also be due to an unbalanced crystal lattice. Whatever the basic cause may be, one can picture the individual ultimate clay particle as a very complex anion. Therefore, to balance its charge, the particle will have the tendency to adsorb the necessary number of cations available in its environment. When such a clay particle is then again dispersed in water, these cations will hydrate and, depending on their valency and degree of hydratability, dissociate to a certain distance from the surface of the particle and thereby build up a diffuse electric double layer and give rise to the formation of a colloidal micelle. Therefore, one may compare the suspended clay particle with a dissociated electrolyte, the size of one of its ions falling within the colloidal range of dimensions. A similar condition exists in the case of soap, where the sodium ion, owing to its hydration,

¹ After this article had gone to press there appeared a paper entitled "Base Exchange of Crystalline Silicates" by H. B. Hendricks (Ind. Eng. Chem. **37**, 625 (1945)), which deserves special attention from those who are interested in the correlation of base exchange with the crystal structure of clay minerals.

will dissociate from the fatty acid ion when the soap is brought into contact with soft water. If, however, hard water is used, then the divalent and less hydrated calcium ions contained therein will exchange for the sodium ions and form the far less soluble calcium soap, as schematically shown in figure 14.

By exactly the same mechanism, the counter ions of the clay particle can be exchanged with ions from the dispersion medium, if the resulting micelle will have less tendency to hydrate and carry a lower charge, or both (74, 100) (figure 15). This reaction is, of course, the more pronounced the more ions of a high

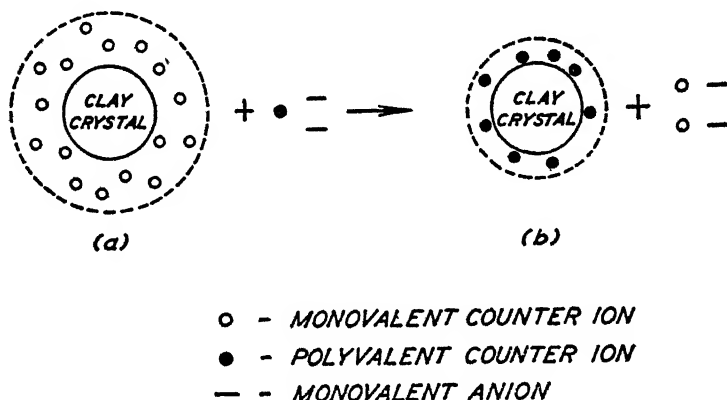


FIG. 15. Schematic representation of the base-exchange reaction of a clay micelle. (a) Clay particle with diffuse electric double layer formed by dissociation of adsorbed counterions; (b) clay particle with diffuse electric double layer reduced in thickness owing to the exchange of the highly solvated monovalent cations for less solvated polyvalent cations.

TABLE 3 (43)

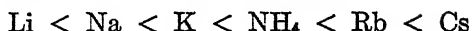
MINERAL	BASE-EXCHANGE CAPACITY
	<i>milliequiv. per 100 g. of clay</i>
Montmorillonite	60-100
Attapulgite.	25-30
Illite....	20-4
Kaolinite.....	3-15

degree of hydration are present in the clay under investigation. The magnitude of this ability to adsorb cations, therefore, depends on the structural configuration of the nucleus of the colloidal clay micelle. It is expressed in milliequivalents of cations per 100 g. of clay. Table 3 gives the exchange capacities for the most important clay minerals.

However, by the same reasoning, clays can also adsorb anions where net positive charges are set up in the crystal lattice, or where the hydrogen of a hydroxyl group is exchanged for a stronger ion, like PO_4^{--} (92).

Quite generally, one can state that ion exchange will follow the Hofmeister or lyotropic series, at least for cations, i.e., the higher the atomic weight of an

ion, the more firmly it will be held by the exchanger. Therefore, the exchange reaction for monovalent ions will follow the series



and for multivalent ions the series



Besides valency and hydration, the size of the exchanging ion is also important, because it is difficult to replace ions which have an apparent diameter of at

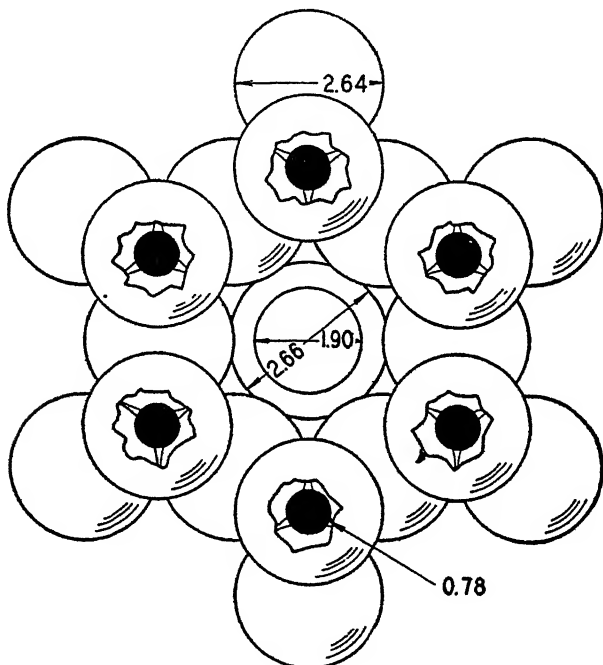


FIG. 16. Hexagonal arrangement of silicon-oxygen tetrahedra in a silica sheet (schematic). The top oxygen atoms have been cut open to show the location of the silicon atoms. The large circle in the center space with a diameter of 2.66 Å. corresponds to the diameter of a potassium atom, the smaller one to a sodium atom. The actual dimensions in Ångström units (1 Å. = 0.1 millimicron) for the different atoms are indicated on the drawing (39).

least 2.64 Å., which is the diameter of the circle one can inscribe into the hexagonal net of oxygen atoms forming the silica sheet (39, 43, 64) (figure 16).

From this one can immediately see why even the dry potassium ion having an apparent diameter of 2.66 Å. is so difficult to replace, and why clays like mica and illite are not readily affected by water. The size of the hydrated exchanged ion is, of course, of importance in regard to the thickness of the formed diffuse double layer, and it will also control the equilibrium exchange, as shown in figure 17.

Base exchange is not, as was assumed for a long time, limited to inorganic cations. Organic cations can be introduced just as well, if they are hydrophilic.

This fact also is the basis for the development of water-resistant clay films, the production and use of which will be discussed in more detail later (41, 43).

The importance of the ion-exchange reaction of clays in explaining the high plasticity, dry strength, thixotropy, dilatancy, and many other properties which are so characteristic of the clay minerals must by now be quite evident, but will become even more so if the reader bears the mechanism of this phenomenon in mind when going over the following sections.

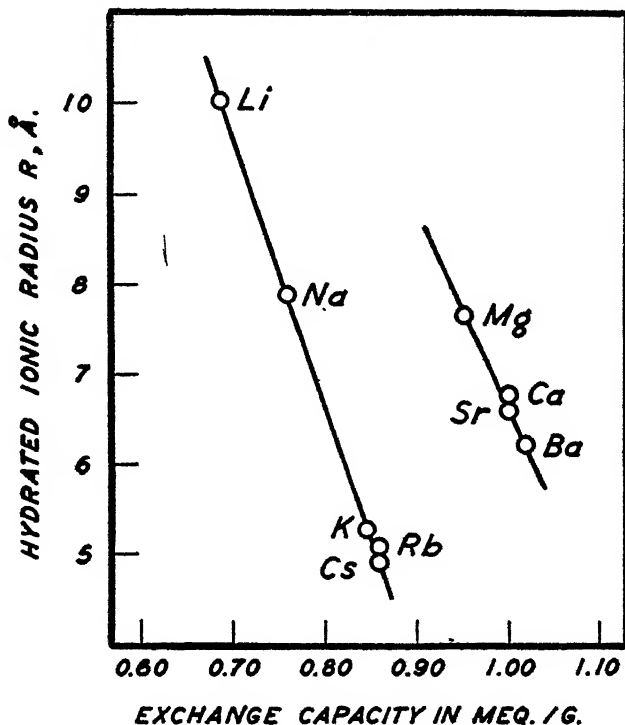


Fig. 17. Radii of hydrated ions *versus* exchange capacity at equilibrium (77)

B. Plasticity

In contrast to a system exhibiting truly viscous flow, one which exhibits truly plastic flow calls for the initial expenditure of force before shear occurs. Therefore, in a truly plastic system some force must first be overcome, which, owing to its influence, prevents the proportional relationship of applied force to shear, as is typical for a truly viscous system. The point at which this relationship is finally established is known as the "yield point". It constitutes the customary measure for plasticity. In clay systems, plasticity is the result of the attraction and repulsion forces set up between the colloidal clay micelles and the ions in the dispersion medium.

In the specific case of kaolinite, hydroxyl ions may be preferentially adsorbed (60). The kaolinite particle consequently takes on a net negative charge, and

an attraction is set up between the kaolinite particle and any positive charges in its environment. The repulsion forces that are associated with the colloidal micelle (46) originate with the counter ions which swarm about the colloidal particle in an effort to neutralize its surface charge. These counter ions complete the diffuse double layer, and, although they may be any cation, the amount of active repulsive force in the system depends on the type of cation serving as the counter ion (60).

In the preceding discussion, the importance of the attraction and repulsion forces connected with the structure of colloidal micelles has been pointed out. It is, therefore, possible to discuss the cause of plasticity on the basis of these fundamental principles.

The most important single cause for the yield value of the kaolinite-water systems probably is the diffuse electric double layer associated with the solid particles or nuclei.

Kaolinite, according to its crystallographic classification, belongs to the monoclinic system and it displays good cleavage. In the formation of fragments, the fracture occurs along the basal cleavage plane and normal to it, forming thin hexagonal platy particles, but fracture along the cleavage plane does not cause the rupture of primary valence bonds. In a fracture parallel to the *c*-axis of the crystal unit, however, it becomes necessary to break the bonds between Si and O, Al and OH, or O (39, 81). These broken bonds serve as the basis for the preferential adsorption of hydroxyl ions (39). The fact, moreover, should not be overlooked that these hydroxyl ions may be bonded by dipolar bonds to the oxygens of the basal silica sheet. In order to set up a negative charge on the particle, the location and mechanism of the attachment of anions on the surface of the particle is, as has been previously stated, irrelevant. The hydroxyl ions thus adsorbed carry with them water molecules (hydration) which make up part of the water hulls. The particle and the cations which swarm about give rise to the Gouy-Freundlich diffuse double layer.

The crystallographic arrangement of the ions or atoms in certain substances causes the ions located on the surface, corners, and edges to be unsaturated and gives rise to the preferential adsorption of oppositely charged ions (39). A consideration of the plastic properties of any mineral must, therefore, include a careful analysis of the characteristics of the diffuse double layer formed by the particular substance under question. This analysis is highly important because, if the plasticity of a substance depends on the particular attraction forces between unsaturated valence bonds and the adsorbed ions, complete information about all of the ions in the system should furnish data relating to its plastic properties.

The size of the particle exhibiting plasticity is of interest only insofar as it affects the attraction forces between the adsorbed ions and the unsaturated valence bonds of the particle. Inasmuch as the limit of size assigned to a colloidal particle is arbitrarily set at a value where surface forces predominate, it is this size which governs the dimensions of the particles exhibiting plasticity. According to the preceding statements, for example, plastic flow and plasticity

of suspensions are colloidal phenomena, and particles of colloidal dimensions, therefore, are obviously necessary in the system to permit this property or any colloidal property to manifest itself. Depending, then, on the nature of the material (a shape factor must be introduced), the size of the particle and its relation to the total surface area must be considered.

In the case of kaolinite, an average equivalent spherical diameter of less than 4 microns appears to be necessary before plastic properties become evident (100). Such an equivalent size is far above the arbitrary value set for the upper limit of the colloidal range. Kaolinite particles, however, exist in the form of plates, their thickness being well within the colloidal range. The surface along this particular dimension is sufficiently active to cause the colloidal phenomenon of plasticity to begin to manifest itself.

In the case of fibrillar particles, two dimensions may lie within the colloidal range, but the third one need not. In the corpuscular system, all three dimensions of the particle must be within the colloidal range. The factor of size is, therefore, important, and careful consideration must be given to this variable in any study of colloidal characteristics.

The plastic properties of the clay mineral attapulgite have been found to be in full accord with its fibrous, crystalline structure (74).

The term "bentonite" refers to a rock formation composed largely of members of the montmorillonite group of clay minerals. Most of these minerals, furthermore, do not, as already pointed out, conform to the ideal structure, and lattice substitutions frequently occur. The crystal units of montmorillonite are only loosely held together by weak O—O bonds. The sodium ions are primarily adsorbed on the surfaces of the silica sheets. When hydration occurs, these ions pry the particles apart and form double layers of appreciable thickness. The clay exhibits a high degree of swelling.

In the formation of bentonite particles, fractures parallel to the *c*-axis, similar to those occurring in the kaolinite crystal, seldom take place. On these surfaces a diffuse double layer, identical with that obtained with kaolinite, can theoretically be formed, that is, first a layer of preferentially adsorbed hydroxyl ions and then the counter ions in a diffuse arrangement. The presence of hydrogen as the counter ion, all other factors being constant, would give the greatest attraction force at the edge of the water hull, and therefore the greatest plasticity. The least plasticity should be exhibited when sodium ions serve as counter charges for the preferentially adsorbed hydroxyl groups.

Although no detailed systematic studies of the plasticity of bentonites have been reported, it is reasonable to assume that their results will be similar, at least in principle, to those for kaolinite. It must naturally be remembered that the particle structure, location, and cause of net charges on the particle surface and, consequently, the distribution of counter ions are not the same, and that the average size of the bentonite particles is smaller than those of kaolinite. There seems to be sufficient evidence, however, to assume that a hydrogen bentonite will be the most plastic and a sodium bentonite the least plastic.

C. Dispersion

The development of the concept which considers the individual clay particle as a colloidal micelle has given the real basis for the understanding of the reactions which control flocculation, deflocculation, and dispersion of clay suspensions. From the foregoing discussions it is evident that these phenomena depend on the ion distribution in the diffuse double layer surrounding every particle or, to state it more concretely, on the ζ -potential of the clay micelle. Thus, any ion which will cause a reduction in the electrokinetic potential will tend to decrease the stability of the suspension, whereas ions which aid in building up the diffuse ion double layer will act as stabilizing or dispersing agents. This approach can best be explained by applying schematic drawings, as in figure 18 (15). Sodium phosphates, tannates, and several other organic sodium compounds have proven to be very effective deflocculating or dispersing agents.

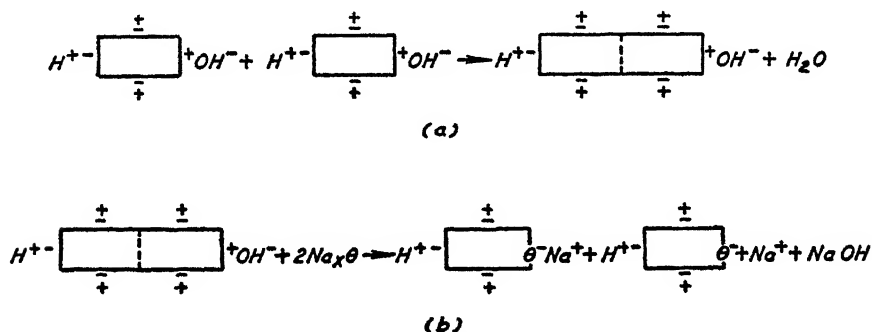


FIG. 18. (a) Condensation of kaolin particles. (b) Dispersion of flocculated clay particles by the addition of a dispersing agent, represented by $\text{Na}\theta$. (Na = sodium; θ = anion.)

D. Dilatancy

This phenomenon was first discussed by Osborne Reynolds (85), when he described how quicksand or an ocean beach at ebb tide will appear moist when at rest, but become quite dry and crumbly when trodden upon. Very little attention was paid to this phenomenon, which frequently has been confused with thixotropy, until it was found that it is not limited, as originally assumed, to spherical particles like starch (80), but that particles of platy shape, like very finely ground mica (48), quartz, glass, porcelain (19), or powdered slate (21) also give rise to a dilatant system, if the suspension is subjected to rapid deformation by stirring, application of pressure, or the like (48).

Dilatancy has so far only been observed with lyophobic suspensions, and the lower the stability of the dispersion, the more pronounced it becomes. Furthermore, only such systems have exhibited dilatancy whose particle size is not smaller than the upper limit of the colloidal range of dimensions. The best explanation for this phenomenon is the one offered by Freundlich (19). When the system is at rest, the particles are independent of each other, thus resulting in

closely packed sediments or gels. If an external force is applied to such a system, it causes unequal distribution of the particles and eventually agglomeration results. Local piling up and the formation of cavities in other places occur. The free dispersion medium is sucked into these, and the entire system will behave as if it were dry (figure 19). When the force is removed, the particles will again take up their equilibrium position in accord with their electrokinetic condition, and the system will revert to its uniform, moist or wet appearance.

Dilatancy can be destroyed either by reducing the concentration of the dispersed phase, or by increasing the ζ -potential of the dispersed particles, or by coating them with a protective colloid. A better understanding of this up to now rather neglected phenomenon will assist in reducing many so far unexplained failures in any scientific work or industrial process in which hydrophobic clay suspensions are used or encountered, as for example in ceramics, oil well drilling, and soil conservation.

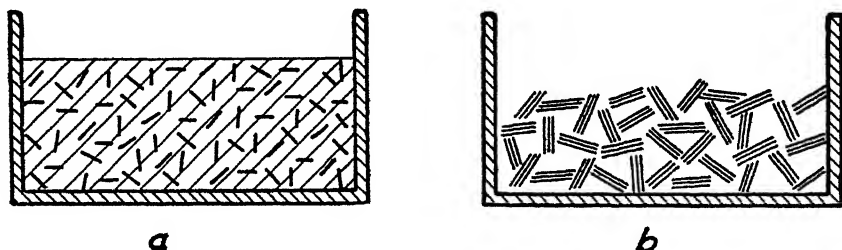


FIG. 19. Dilatancy: (a) system at rest; (b) system when subjected to pressure or mechanical agitation.

E. Thixotropy

In 1923 Szegvari and Schalek noticed that a colloidal iron oxide sol will set to a gel upon the addition of electrolytes and that this gel can be liquefied simply by shaking. If then allowed to rest it will again set to a gel (93). Péterfi (83), who somewhat later noticed the same phenomenon in cell protoplasm, coined the name, deriving it from the Greek words *thixis* (thixis) meaning *the touch*, and *trépo* (trepo) meaning *to change*. H. Freundlich, to whom we owe the first and only existing summarizing treatise of this interesting colloidal phenomenon (18), was also the first to find that bentonite, if dispersed in water at a concentration of about 12 per cent, will give a thixotropic gel (17). Shortly thereafter it was discovered that dialyzed bentonite did not exhibit thixotropy, but that this phenomenon could be induced by the addition of alkali hydroxides to the dispersion medium. More detailed discussions of the effect of electrolytes on the thixotropy of bentonite suspensions of high concentration have been published by Freundlich and his coworkers (24, 25).

The first ultramicroscopic study of thixotropic bentonite sols was made by Hauser, who also proved that a thixotropic bentonite gel can be obtained at concentrations of only 0.26 per cent if the right amount of electrolyte is added.

Furthermore, it was demonstrated that, upon addition of electrolyte to a dialyzed bentonite sol, first the translatory and then the rotary component of the Brownian molecular motion stops (34). These results made it probable that particle size and the corresponding development of diffuse ion double layers influenced thixotropic gelation. This was proven by subjecting dispersions of crude bentonite to fractional supercentrifugation (50) and determining the thixotropy of various particle sizes. It could be shown that thixotropy became more pronounced the smaller the particle size. Particles with an average equivalent spherical diameter of 15 m μ would yield a thixotropic gel at concentrations as low as 0.05 per cent (51). This, coupled with ultramicroscopic (42) and more recently electron-microscopic observations and the fact that no change in volume can be detected when the sol changes to a gel, that preferentially anisometric particles yield truly thixotropic systems, and finally that the electrokinetic condition of the colloidal micelle is of primary importance, permit us to draw the following conclusion: Clay dispersions exhibiting thixotropy are characterized by such distribution of ions in their diffuse double layer, resulting either from adsorption by added electrolytes or from surface dissociation, that the particles with their solvated hulls will, if left undisturbed, take on equilibrium positions. However, if disturbed, the solvated hulls are disrupted and the particles can then freely move around. This concept is substantiated by the sudden change in light transmission when a bentonite sol sets to a gel (35), and is also in line with Langmuir's mathematical treatment of thixotropic systems (65), which concludes that the particles in the gel are placed like ions in a crystal and lie at considerable distance (up to 5000 Å.) from each other. They are locked into their places by the balance of far-reaching attraction forces and appropriate repulsion forces set up by the interplay of the surface ions of the clay crystal and the free ions in the dispersion medium (19). This theory, based on actual observations, was, therefore, substituted for the previous so-called mechanical theory, which assumed that the clay particles touch each other in completely random, three-dimensional orientation (6).

Thixotropy has also been found with Solnhofen slate, famous for its petrifactions of such delicate structures as jellyfish (21). The thixotropy of quicksand must also be attributed to the presence of colloidal clays of high base-exchange capacity (22).

F. Rheopexy

The phenomenon of rheopexy (derived from the Greek words $\rho\acute{\epsilon}\omega$ (reo) meaning *to flow*, and $\pi\eta\kappa\tau\acute{o}\varsigma$ (pectos) meaning *solidified or curdled*) has so far only been observed with a few systems which are known to be thixotropic. Freundlich and Juliusburger (23) found that the setting time of thixotropic suspensions of colloidal gypsum and of vanadium pentoxide could be drastically reduced if the test tube containing the sol was slowly rolled back and forth between the palms of the hands. The same result was obtained by tapping the tube on a table at regular intervals (20), or even better on a rubber pad (49), or simply by swinging it in a circular plane (48), or moving it like a pendulum at an amplitude of the

oscillations of 15–20° from the vertical position (51). Hauser and Reed (49), using fine-particle-size fractions of bentonite, obtained by supercentrifuging bentonite sols, were the first to prove, contrary to previous statements (23), that such bentonite sols were not only thixotropic, but also pronouncedly rheoplectic.

The difference in thixotropic and rheoplectic setting time and the influence of particle size is shown in figure 20 (51). The same effect can be obtained by adding to the negatively charged hydrogen bentonite a positively charged colloid, as, for example, iron oxide. If one adds 0.025 g. of it to 1 g. of hydrogen bentonite (dry weights), a gel results which has a thixotropic setting time in excess of 130 hr., but only 2 min. of rheoplectic setting time (51). In view of the presence of iron oxide in many clay deposits, this phenomenon deserves more attention than it has so far received, particularly in geology, petrography, and

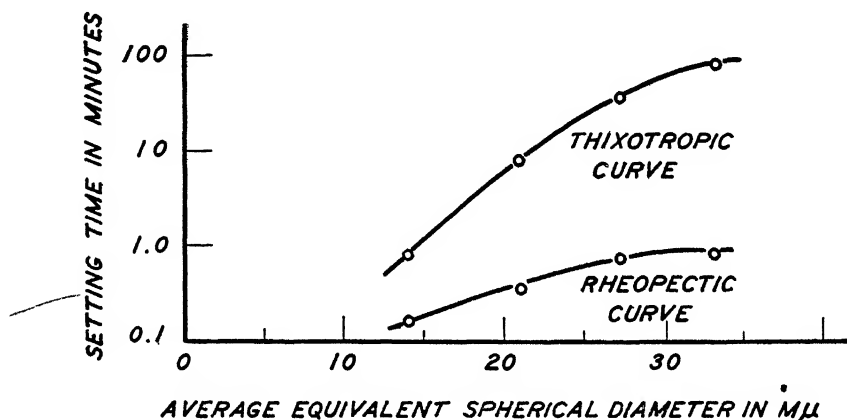


FIG 20. Influence of particle size on setting time: 0.85 per cent bentonite; 76.5 millimoles potassium hydroxide per liter; temperature, 25°C (51).

soil chemistry. The only other reference to rheopexy in clay systems is a statement by Freundlich (19) that the finest fractions of Solnhofen slate exhibit this phenomenon. This discovery deserves special attention, because it offers the most plausible explanation for the formation of the perfect petrifications of jellyfish for which these slates are well known. Similar findings in other parts of the world have always perplexed geologists (21).

Whereas thixotropic systems have been obtained from all possible types of colloidal dispersions, truly rheoplectic systems seem to be limited to laminar systems, for which the clays are perfect examples. Besides this, rheoplectic clay systems are limited to those clays which also permit production of thixotropic systems.

Whereas the setting up of a thixotropic gel does not call for any preferential alignment of the dispersed particles, ultramicroscopic studies of rheoplectic systems indicate that the rhythmic motion applied to the sol orients and aligns

the platy particles and thereby accelerates their taking up the equilibrium positions already referred to when discussing the phenomenon of thixotropy.

Figure 21 represents a schematic interpretation of such alignment.

G. Stream double refraction

If one views a colloidal sol containing anisometric particles, preferably of rod or plate shape, between crossed Nicol prisms or crossed Polaroid sheets against a strong light source, no double refraction can be noticed. The reason for this is that the colloidal particles are subjected to constant Brownian molecular motion and are thereby prevented from taking up any preferential orientation. However, if such a sol is caused to flow, the disperse particles will always tend

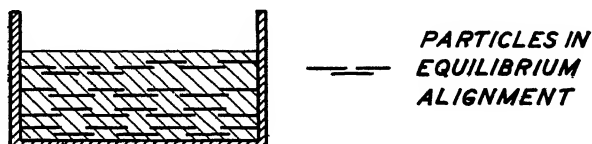


FIG. 21. Alignment of particles in a rheopectic system after setting caused by tapping container on flat table.

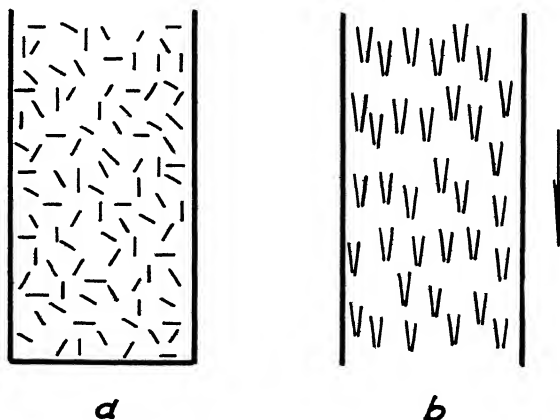


FIG. 22. (a) Complete disorder of platy colloidal particles dispersed in a stagnant liquid; (b) alignment of platy colloidal particles dispersed in a flowing liquid.

to orient in such a way that the planes of the disks or plates lie tangent to the surface of cones whose apexes point in the direction of flow (65, 70), and such a system will then exhibit the phenomenon known as stream double refraction or birefringence (figure 22).

Since most clays are composed of platy particles, it is only logical to assume that they must exhibit this phenomenon. This is actually the case, but permanent effects can only be expected if truly colloidal clays are used, because only then can one eliminate sedimentation of the disperse phase (3). Langmuir's (65) studies of polarization of light passing through a flowing bentonite sol indicate that the clay particles are thin flat plates of irregular shape, their lengths

being only somewhat greater than their widths. A purified, monodisperse bentonite sol of 1 per cent concentration, produced by supercentrifuging and containing particles ranging in apparent spherical diameter between 15 and 50 $m\mu$, proved particularly suitable (42) for studies of stream double refraction, because such a sol is quite clear to the eye, has practically the same viscosity and surface tension as water, and exhibits pronounced birefringence at extremely low rates of flow and for any temperature up to the boiling point of water (44).

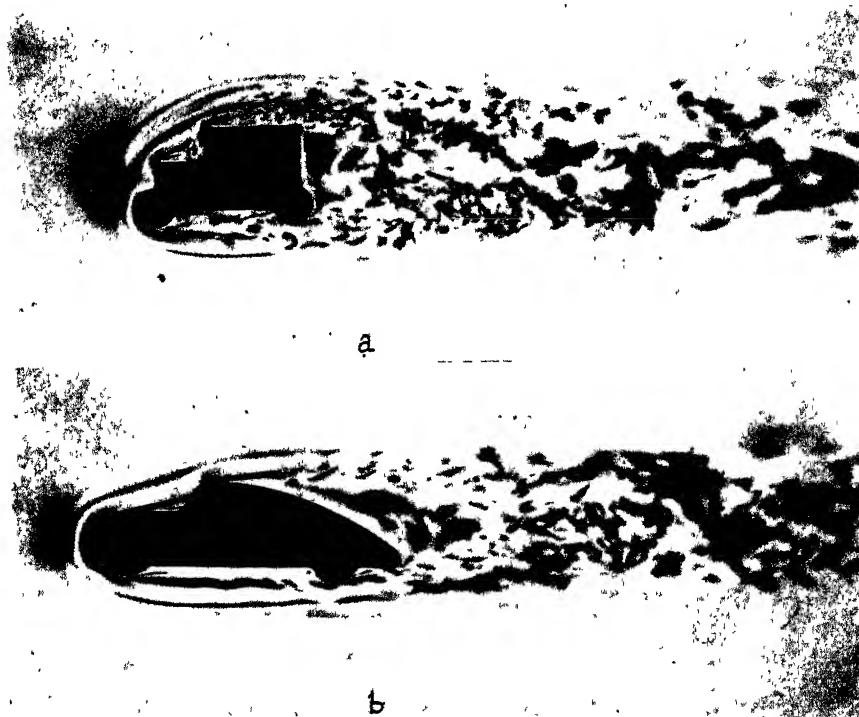


FIG. 23. Flow patterns, as shown with circular polarized light, around models depicting (a) the old-fashioned *versus* (b) the more modern streamlined body of an automobile traveling at high speed (45).

This property of colloidal clays has permitted the development of a very precise technique for studying flow patterns visually. Experimental measurements of the amount of double refraction caused by different values of velocity gradients enable one to interpret quantitatively the patterns in regions of laminar flow or even turbulent flow conditions. Figure 23 gives an idea of the applicability of this technique in engineering (45). War conditions do not permit as yet the discussion of further developments of this technique, made during the last few years in the fields of aeronautics and shipbuilding. However, the method has also been very successfully applied in the study of firebox design of railroad locomotives and other railroad engineering problems (66), and it is finding

increasing use in the visual study of the efficiency of water turbines, condensor scoops, and other hydraulic machinery (33).

Similar effects are obtained by applying an electric field to the system. This method permits optical study and measure of inhomogeneous electric fields in liquids comparable to studies of photoelasticity of solids (43, 76).

V. APPLIED COLLOID CHEMISTRY OF CLAYS

From the foregoing discussion of the most important colloidal phenomena and properties of clays, it must be evident that not only can their industrial use now be put on a far more intelligent basis,² but new applications have come and will keep on coming to light. In the following pages those fields of science and technology will be briefly discussed in which the colloid chemistry of clay minerals has already proved its value and which are considered to be most important. Many other uses, as for example in the production of emulsions, in the bonding of foundry sands, in insecticide and fungicide or bactericide sprays, and in lubricants, have not been specifically referred to, because the use of colloidal clays for these purposes must be self-evident when their colloidal properties and phenomena are taken into consideration.

A. *Ceramics* (30, 47, 78)

Of all the industries in which clay minerals are of predominant importance, the ceramic industry is, of course, the dominating one. It is the property of plasticity, so characteristic of clays, which permits their forming to practically any desired shape. However, not all clays exhibit this property to the same degree, and therefore pottery was for centuries an art which had to rely on trial and error tests before the suitability of a clay or mixtures of different clays could be established. With the comparatively recent development of the concept of the clay particle as a colloidal micelle it has not only been possible to offer a better understanding of such properties as plasticity, dry strength, shrinkage, and others, but it has also been clearly shown that the change of ceramics from an art to a science was at hand. By applying the new concept and its many ramifications intelligently, this change has been accomplished comparatively recently. Not only have we now a clear picture of the phenomena so important in ceramics but, knowing the reasons for their occurrence, we have also learned to control them, to change them when needed, and to apply them in such a way and to just such a degree as is most profitable for the purpose. As can be readily seen from the foregoing discussions, ceramics depends on modern colloid chemistry far more than is generally assumed, and recent developments in this field of applied colloid chemistry have offered the best proof for this point of view.

B. *Agriculture* (61)

Since clay is one of the fundamentally most important substances in soils, it is understandable that more attention is now being paid to clay research in

² C. G. Harman and C. W. Parmelee have just published a paper, "Fundamental Properties of Raw Clays Influencing their Use" (J. Am. Ceram. Soc. **28**, 110 (1945)), which offers further proof for the importance of surface or colloidal phenomena in clay-water systems.

connection with agriculture than ever before. The clay content and the type of clay present in the soil control above all its moisture content, its ability to supply water, its acidity, its tilth, and its general properties. Our increased knowledge of the colloid chemistry of clays and of its application in agriculture is already contributing its share for a deepening of our knowledge in the domain of soil science.

C. Soil engineering

The ion-exchange reaction of clay minerals has also become of importance in the domain of construction engineering. The application of this colloidal phenomenon for reduction of soil permeability or seepage is so far the most striking evidence for the value these new concepts have in fields where the application of chemistry was unknown only a short time ago.

Take, for example, the fresh-water lake on Treasure Island in San Francisco Bay. When built, the seven-acre lagoon showed a drop of 1 in. per day of its water level, which made it questionable if the lagoon could be maintained. The answer was simple. The sea water from the bay was pumped in and the pervious natural soil was transformed by ion adsorption and exchange into a non-pervious sodium soil. The sea water was removed and the lagoon filled with fresh water. The loss in level due to seepage dropped to 0.1 in. or even less per day (67).

The importance of a combination of our knowledge of the structure, chemical composition, and colloid-chemical considerations of clays has also proved its value in soil stabilization. This is of particular importance in highway engineering, construction of dams, and flood control (101).

D. Drilling muds

As has already been pointed out, montmorillonite, the major component of bentonite, exhibits the phenomenon of thixotropy to a very pronounced degree. It is this property which makes suspensions of this type of clay so well suited to the use to which so-called drilling muds are being put. The purpose of the mud is to hold the rock fragments obtained during the drilling of an oil well in suspension if the drill is at rest so that they do not sediment and form a cake around the drill and shaft. However, when the drill works it should find a minimum of resistance, and the liquid carrying the rock fragments or sand should have a low viscosity, so that it can be easily removed from the bore hold by pumping. A dispersion of clay whose repulsive and attractive forces have been so adjusted that a thixotropic system is created fulfills these requirements (43). Such drilling muds are also used to seal off formations traversed during the drilling operations, so that liquids or gases contained therein cannot interfere with the drilling operation or with the use of the oil well when put into operation (43).

E. Films and plastics

One of the most interesting and recent discoveries in the field of applied colloid chemistry of clays is their use in the production of coherent, self-supporting, and flexible films and their applicability as the basic material for mold-

able plastics (14, 41, 43, 94, 96). These films have been termed "Alsifilm" (*aluminum silicate films*).

If a thixotropic gel of grit-free montmorillonite, preferably one exhibiting high base-exchange capacity, is spread onto a smooth surface and subjected to careful drying, a film will form which, being coherent and self-supporting, can be easily removed from its support. By using conventional-type spreading machines, the process can be made continuous and the formed film wound up on a mandrel just as in the production of paper or other organic film or sheet-forming substances. If one observes the transition from the gel state to the coherent dry film with the use of an ultramicroscope of the Ultropak type (38), one can see how the clay ultramicros align themselves during evaporation and form intersecting and interweaving threadlike aggregates until a micro-fabric

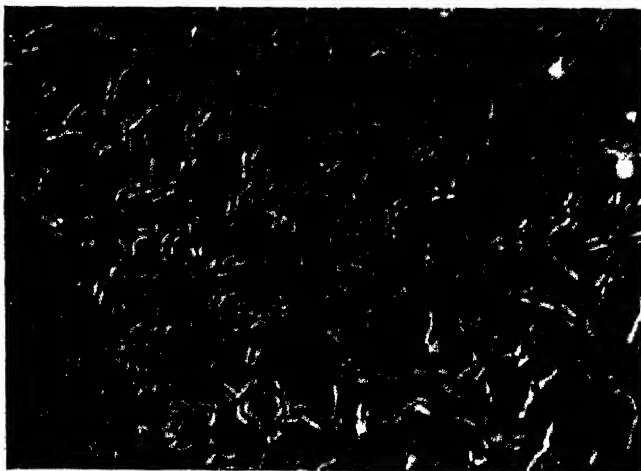


FIG. 24. Ultropak microphotograph of the structure of a crude montmorillonite film (Alsifilm) after being heated to 250°C. (42).

structure, as shown in figure 24, results. X-ray diagrams of such films taken parallel and at a right angle to their plane yield a typical Debye-Scherrer diffraction pattern of the clay mineral in the latter case, but a fibre pattern in the former. This proves that all the particles have aligned with the same crystallographic axis parallel to the support (42).

These films still exhibit all the properties of the original clay. In contact with water they will eventually again revert to a sol. Marshall and his coworkers have shown that such sheets made from the clay minerals montmorillonite or beidellite are of great value when used as membrane electrodes in the study of cation activities with a precision within 5 per cent at pH values above 4 (71, 72, 75).

The fact that the crude clay film is not resistant to humid atmospheres or moisture, even if compounded with water-resistant fillers or fibres like paper pulp (41), drastically limited its use. However, the fact that Alsifilm still permits base exchange has made it possible to transform the swellable and

redispersible film into an absolutely non-swellable condition by binding the ultimate clay particles to each other by the use of the proper type of ions (41, 42, 53).

The result is a film which is no longer affected by moisture. Its structure is comparable to that of muscovite and might, for reasons schematically shown in figure 25, be called synthetic mica (103).

Of particular interest is the fact that base exchange can also be accomplished with ions carrying organic radicals, as, for example, organic ammonium complexes which, after drying, can be condensed or even polymerized. Thus we have before us an interesting link between the inorganic and organic worlds.

Clay films which have been rendered water insoluble are excellent electric insulators, approaching the best types of mica in this respect. They are oil resistant and are not affected by organic solvents, fats, and waxes, and should, therefore, find interesting application in the field of packaging.

If paper is coated with such coherent clay films, its surface will become glossy and very resistant to all kinds of destructive influences.

The same inertness is also one of the outstanding characteristics of molded goods made from clays which have been base exchanged as described. By using appropriate organic exchange ions, another interesting link between ceramics and plastics has been established.

F. Thixotropic rubber compounds

The viscosity of the natural milk sap or latex of the rubber tree is very low if one compares it with the viscosity of a solution of rubber in an organic solvent, even if the concentration of rubber is many times that of the organic solution. Although this low viscosity has many advantages for the use of latex, it is a serious drawback wherever too great a fluidity of the system is disadvantageous. It is much easier to squirt a latex compound through a fine nozzle than a highly viscous solution of rubber in benzene, and the concentration in rubber will be much greater for any given volume. But, owing to its low viscosity, the latex would tend to spread out when deposited on a surface, as in the ends of container closures and the like. However, if a thixotropy-inducing clay dispersion is added to the latex, the compound will set up to a gel as soon as the dispersion has come to rest, and spreading will be avoided. This has made it possible to produce latex compounds which can be easily applied to the groove in a can end. The thixotropy of the compound will cause it to set up as soon as it comes to rest in the can end. It is then dried and the resulting rubber ring used as the seal for the food container (9).

G. Thixotropic adhesives

In the production of adhesive pastes containing rubber as the constituent causing adhesion, as used in the shoe industry, it is advantageous to have a paste which can be easily applied by brushing but which, as soon as applied, will set and thereby avoid flow or uneven distribution. Here again the application of thixotropy has proved of extreme value. Such adhesives (8) are composed of rubber latex to which a bentonite dispersion has been added.

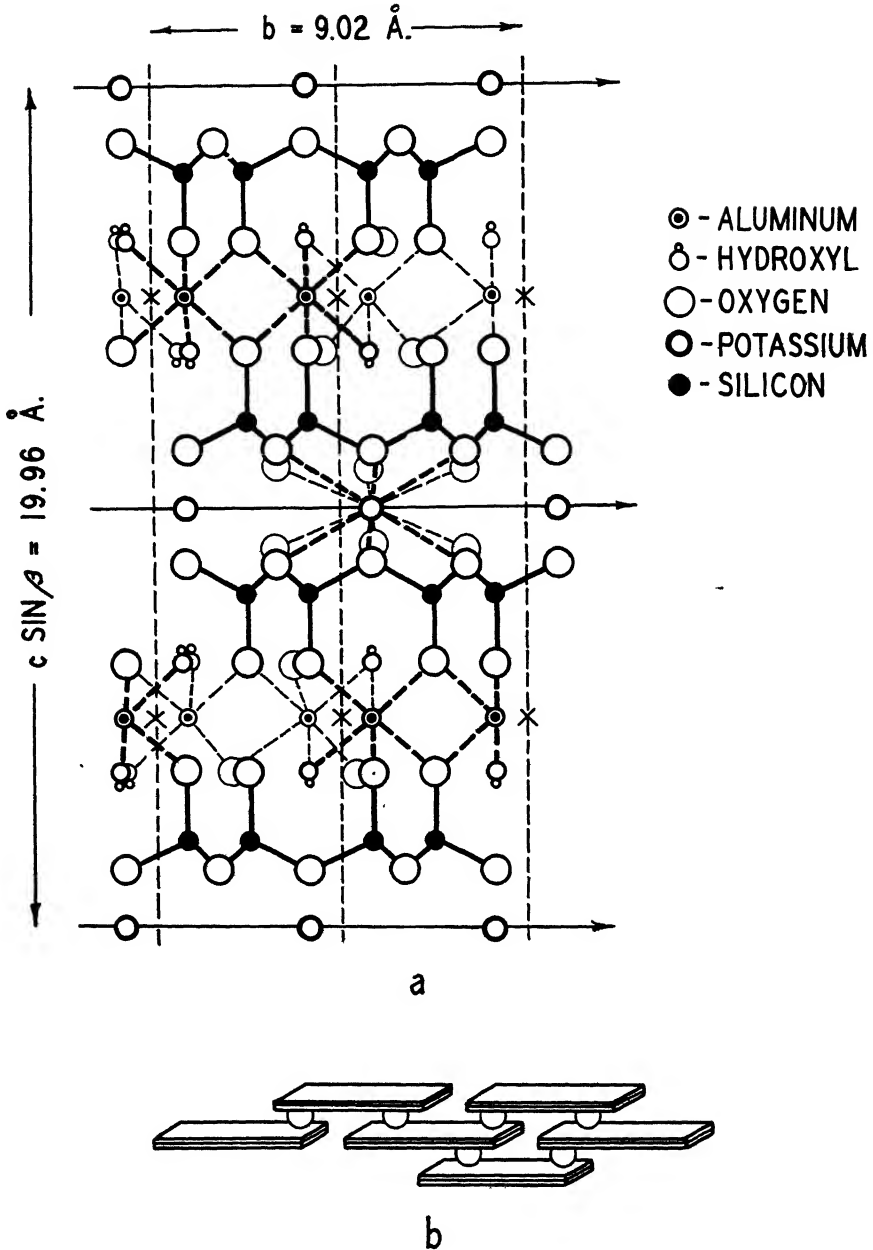


FIG. 25 (reference 42). (a) Schematic drawing of the structure of mica (muscovite) projected at a plane normal to the a -axis. The double sheets are seen with potassium atoms between them surrounded by twelve oxygen atoms (from W. L. Bragg: *Atomic Structure of Minerals*, p. 207, Cornell University Press, Ithaca, New York (1937)).

(b) Probable bonding of unit layer parcels of montmorillonite by large cations, producing water-resistant clay films.

This adhesive, therefore, when brushed onto the leather will flow readily, but as soon as the brushing is stopped it will, owing to the thixotropy of the bentonite, set and form a stable adhesive layer.

H. Water softening (97)

A very important application of clays which possess the property of ion exchange is in water softening. If hard water, which contains calcium and magnesium salts, is percolated through a bed filled with clay which carries sodium ions as its exchangeable counter ions, they will, as was shown in figure 15, be replaced by the calcium or magnesium ions until all the accessible free negative valencies of the clay particles are compensated by the concentration and valency of the added cations. Then, by applying the mass-action law, i.e., by using a high concentration of a concentrated solution of a sodium salt, like brine, one can regenerate the clay and again form a sodium clay.

Another technical application of the ion-exchange reaction can be found in the production of hydrocyanic acid from formamide, where natural aluminosilicates are used as dehydrating agents (56). Their application as nuclei for catalysts, by introducing appropriate metallic ions, has also been suggested (88).

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³ Note added in proof: Since this article was submitted for publication a few papers have appeared which should be mentioned to keep this review up to date.

C. C. Ross has published a paper entitled "Minerals and Mineral Relationships of the Clay Minerals" (*J. Am. Ceram. Soc.* 23, 173 (1945)), in which he discusses in great detail the properties of colloidal clays and the possibilities of applying base-exchange reactions for specific purposes.

S. Sussman and A. B. Mindler have published a paper entitled "Ion Exchange—An Old Process Finds New Uses" (*Chem. Industries* 56, 789 (1945)), in which a number of new applications for ion-exchange materials are discussed.

S. B. Hendricks has published a paper entitled "Base Exchange of Crystalline Silicates" (*Ind. Eng. Chem.* 37, 625 (1945)), in which crystallographic properties are correlated with the base-exchange capacity of the various clays.

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THE ALKYLATION OF ALKANES

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CONTENTS

I. Introduction.....	323
II. Catalytic alkylation of alkanes.....	324
A. Aluminum chloride.....	324
1. Alkenes.....	324
2. Alkenyl halides.....	327
B. Aluminum chloride and alkali chloride.....	327
C. Aluminum bromide.....	331
D. Aluminum bromide and aluminum chloride.....	333
E. Zirconium chloride.....	333
F. Boron fluoride.....	333
G. Hydrogen fluoride.....	335
1. Alkenes.....	335
2. Alkyl fluorides.....	340
H. Sulfuric acid.....	340
III. Thermal alkylation.....	349
A. Alkenes.....	349
B. Methyl radicals.....	361
IV. Summary of the products of alkylation.....	367
V. Mechanism of alkylation.....	369
A. Catalytic alkylation.....	369
B. Thermal alkylation.....	396
VI. References.....	398

I. INTRODUCTION

The alkylation of alkanes was discovered in 1932 by Ipatieff and Pines in the research laboratories of the Universal Oil Products Company (24a). This fundamental reaction is the basis of three commercial processes for the production of *alkylate*, a primary component of 100-octane aviation gasoline. These processes use aluminum chloride, hydrogen fluoride, and sulfuric acid as catalysts for effecting a juncture of isobutane with propene, butenes, pentenes, or octenes. Ethene has been used as an alkylating agent in the presence of aluminum chloride.¹ The three processes were producing alkylate at the rate of 5,500,000 gallons a day during the war period.

In 1936 the alkylation of isobutane by isobutene (2-methylpropene) to form 2,2,4-trimethylpentane was confirmed on thermodynamic grounds (43). Previously some chemists were dubious about the occurrence of a reaction requiring relatively low temperatures and involving alkanes believed to be chemically inert and to have low reaction velocities. Thermodynamics is not concerned with the catalyst or time required for the reaction.

The present review considers the available literature, exclusive of patents, on the alkylation of alkanes. Patents will be reported upon at a later date. Seven

alkylations with three normal alkanes (propane, butane, and hexane) and fifty alkylations with three branched-chain alkanes (isobutane, isopentane, and 2,2,4-trimethylpentane) are discussed in the following pages. Of these fifty-seven alkylations, seven use aluminum chloride; four use aluminum chloride plus alkali chloride; four use aluminum bromide; one uses aluminum bromide plus aluminum chloride; one uses zirconium chloride; six use boron fluoride; eight use hydrogen fluoride; twenty use sulfuric acid; and one uses phosphoric acid as catalyst. Five alkylations are thermal reactions. Isobutane has been alkylated by fifteen alkenes, one alkadiene, four alkyl halides, one dialkyl sulfate, and two alkenyl chlorides. Isobutane reacts with ethene to yield mainly 2,2-dimethylbutane (thermally) or 2,3-dimethylbutane (catalytically, except in the presence of sulfuric acid). Mechanisms of alkylation proposed by eight groups of investigators are presented.

II. CATALYTIC ALKYLATION OF ALKANES

The chief catalysts for the alkylation of alkanes have been found to be aluminum chloride or bromide and modifications thereof, zirconium chloride, boron fluoride, hydrogen fluoride, and sulfuric acid. These catalysts lead to the formation of typical alkylates whose production and composition will next be considered.

A. ALUMINUM CHLORIDE

1. Alkenes

Alkanes from butane to dodecane have been alkylated by alkenes in the presence of aluminum chloride (30). The alkylations occurred at 25–40°C. under 1–15 atmospheres pressure, producing colorless alkylated alkanes and a brown lower layer composed of aluminum chloride in combination with high-boiling unsaturated hydrocarbons. Experimental data were given for the alkylation of isobutane and *n*-hexane by ethene under mild pressure conditions (table 1).

The data of table 1 demonstrate that isobutane and *n*-hexane reacted with ethene to form a series of ethylated (or rearranged methylated) hydrocarbons. All of the alkylations were complicated by side reactions yielding alkanes with an odd number of carbon atoms. Three side reactions, namely, "autodestructive alkylation" of alkanes (27), conversion of ethene or other alkenes into alkanes (29), and formation of aluminum chloride-hydrocarbon complexes, were mentioned as occurring simultaneously, though to an extent varying with the experimental conditions. "Autodestructive alkylation" of alkanes consists in the splitting of an alkane into a lower alkane and an alkene that reacts with another molecule of the initial alkane to form a higher alkane; it causes the formation of alkanes with more and less carbon atoms than the primary alkylate. Conversion of ethene into by-product alkanes appears to involve a polymerization of ethene with subsequent hydrogenation of the alkene polymers. The requisite hydrogen atoms are derived from the unsaturated hydrocarbons that combine with aluminum chloride to form deep red-brown viscous lower layers. A certain amount of hydrogen chloride appears to be necessary for the occurrence

of an alkylation in the presence of aluminum chloride (24). This hydrogen chloride may be added as such or may be derived from alkyl chlorides or from sludge formation. We presume that one function of hydrogen chloride is to produce monomeric aluminum chloride, i.e., $(\text{AlCl}_3)_1$, both by dissociation of associated molecules of aluminum chloride and by decomposition of aluminum chloride-hydrocarbon complexes. Hydrogen chloride exhibits an ability to

TABLE 1

Alkylation of isobutane and n-hexane by ethene in the presence of aluminum chloride
(Ipatieff, Grosse, Pines, and Komarewsky)

Reaction conditions:			
Isobutane, grams.....	62.3	0	0
n-Hexane, grams.....	0	147.7	150
Ethene, grams.....	95.6	61.3	100-110
AlCl_3 , grams.....	22.4	25.1	30
Anhydrous HCl		"A little?"	
Temperature, °C.....	25	40	25
Time, hours.....	10	15	36
Pressure, atmospheres.....	15 (max.)	1	15 (max.)
Uncondensed gas, grams.....	4.9	0	0.4
Weight upper layer, grams.....	142.0	158.0	225
Weight lower layer, grams.....	31.8	60.5	50-55
Fractionation of upper layer:			
Amount fractionated, grams.....	135	158	220
Main fractions, grams:			
B.p., °C.	Composition		
20- 45	C_8H_{12}	9.9	
25- 50	C_8H_{12}	7.4	4.4
45- 85	C_8H_{14} - C_7H_{16}	49.2	
50- 70	C_8H_{14}	11.5	
50- 75	C_8H_{14}		75.1
70-100	C_7H_{16}	24.3	
75-100	C_7H_{16}		10.85
85-125	C_7H_{16} - C_8H_{18}	25.6	
100-125	C_8H_{18}	24.6	18.15
125-150	C_8H_{20} - $\text{C}_{10}\text{H}_{22}$		12.05
125-160	C_8H_{20} - $\text{C}_{10}\text{H}_{22}$	13.3	
125-170	C_8H_{20} - $\text{C}_{10}\text{H}_{22}$	25.6	
Higher fractions, grams.....	51.6	40.6	94.05

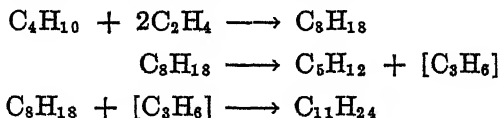
react with or decompose organic complexes containing metal salts, such as aluminum chloride. Another function of hydrogen chloride may be to add to the alkenes present, yielding alkyl chlorides that serve as alkylating agents. Early speculations (16) as to whether the most active form of the catalyst is $(\text{AlCl}_3)_1$ or hydrogen aluminum chloride, i.e., HAlCl_4 , appear to have been resolved in favor of monomeric aluminum chloride. It is known that HAlCl_4 , if capable of existence, has a very high dissociation pressure at ordinary temperature (24). Consequently, its formation would serve to maintain an effective supply of monomeric aluminum chloride.

Partly published results of Pines, Grosse, and Ipatieff have revealed that hexanes can be made the major product in the aluminum chloride-catalyzed alkylation of isobutane by ethene under pressure at room temperature (26):

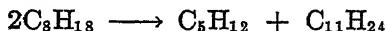
PRODUCT	YIELD
	<i>volume per cent</i>
Isopentane.....	16.0
Hexanes	41.0
Heptanes.....	9.4
Octanes.....	12.3
Nonanes.....	6.5
Higher alkanes	14.8

A subsequent study identified the products formed in the catalytic alkylation of isobutane by ethene at 25–35°C. under a maximum pressure of 10 atmospheres in the presence of aluminum chloride and hydrogen chloride (20). The hexane fraction constituted 45 per cent of the total liquid product and was found by parallel chemical and Raman spectroscopic examinations to contain 10–25 per cent of 2-methylpentane, less than 3 per cent of 2,2-dimethylbutane, and 70–90 per cent of 2,3-dimethylbutane.

Ipatieff (26) advanced the idea that alkylation of C_4 or higher alkanes by alkenes in the presence of aluminum chloride probably involves reaction of the alkane (preferably in branched form) with an aluminum chloride-alkene complex. In the alkylation of isobutane by ethene, taken as an example, the expected products were 2,2-dimethylbutane and a lesser amount of 2-methylpentane. Since the main product among the hexanes was 2,3-dimethylbutane, it was assumed to be an isomerization product of the primarily formed 2,2-dimethylbutane. Other products, such as isopentane and undecanes, were ascribed to the operation of autodestructive alkylation, e.g.,



or



Non-reactivity of propane toward alkylation was ascribed to its lack of a tertiary carbon atom, as well as the impossibility of its isomerization into an isomer containing such an atom. Propane, however, reacts extensively with ethene or propene in thermal alkylation under pressure.

Alkylation of isobutane by propene and mixed *n*-butenes (33 per cent 1-butene and 67 per cent 2-butene) in the presence of aluminum chloride and anhydrous hydrogen chloride was studied at low temperatures, permitting operation in the liquid phase at atmospheric pressure (44). A continuous-operation glass apparatus was designed for regulation of the temperature, contact time, and feed rate. The reaction of isobutane with propene was carried out at -30°C . with a con-

tact time of approximately 4 min. A total of 3230 cc. of liquefied hydrocarbons (23 mole per cent of propene) was brought into contact with 15 g. of aluminum chloride, and hydrogen chloride was introduced simultaneously. The liquid product, which amounted to 650 cc., contained 42 per cent of heptanes (mainly 2,3-dimethylpentane with some 2,4-dimethylpentane, according to Raman spectroscopic analysis (46)) and 20 per cent of decanes. Ninety per cent of the liquid distilled below 220°C. Isobutane and *n*-butenes under similar conditions (-35°C., aluminum chloride and hydrogen chloride as catalyst) gave a liquid product containing alkanes only, including over 60 per cent of octanes and 12 per cent of dodecanes. By means of Raman spectra, these octanes were found to be 2,5-dimethylhexane and 2,2,3-, 2,2,4-, and 2,3,4-trimethylpentanes.

The reaction of isobutane with isopropyl chloride in the presence of aluminum chloride at 40-70°C. does not yield heptanes as the major product. Instead, reduction of the alkyl chloride to propane occurs to the extent of 60-90 per cent; the isobutane furnishes the hydrogen and is converted chiefly into octanes and catalyst complex (53).

2. Alkenyl halides

A study of the condensation of alkanes with alkenyl halides in the presence of aluminum chloride has led to a new method of preparation of haloalkanes as well as the corresponding alkanes (48). By proper choice of reaction conditions, e.g., at temperatures above 0°C., an interaction of 1.5 moles of isobutane with 1 mole of allyl chloride could be obtained, yielding liquid alkanes to the extent of 310 per cent by weight of the propene available from the allyl chloride. At lower temperatures the conversion ends with the formation of 1-chloro-3,4-dimethylpentane and 1,2-dichloro-4,4-dimethylpentane; it was stated that these react with more isobutane at the higher temperatures to form alkanes and hydrogen chloride. Liquid isoalkanes were also formed in the condensation of isobutane with vinyl chloride; about 10 per cent of *tert*-butyl chloride and up to 40 per cent of 1,1-dichloro-3,3-dimethylbutane were by-products of the reaction.

B. ALUMINUM CHLORIDE AND ALKALI CHLORIDE

Isobutane has been alkylated by gaseous alkenes in the presence of pumice coated with double compounds of aluminum chloride and alkali chlorides (10). These alkylations were complicated by a competing polymerization of alkenes and by side reactions forming gasoline-range hydrocarbons with intermediary numbers of carbon atoms. Mixtures of the alkenes with isobutane in excess were passed over the catalysts under pressures of about 1000 psi¹ and temperatures of 154-291°C. Ammonium aluminum chloride (NH₄AlCl₄) was found to be somewhat less active than lithium aluminum chloride (LiAlCl₄) or sodium aluminum chloride (NaAlCl₄); it required a temperature of about 232°C. for any substantial activity. Potassium aluminum chloride (KAlCl₄) was nearly inactive, showing some polymerization of alkene at 316°C.

¹ psi = gage pressure in pounds per square inch.

Table 2 gives the results of two tests in which isobutane and propene were reacted over lithium aluminum chloride on pumice. In each case the products were collected in two parts. Their analyses indicate that the alkylating ability of the catalyst declined rapidly (*cf.* liquid volume percentage of C_7 hydrocarbons and of alkenes therein; see also figure 1). The catalyst after use was found covered with a heavy carbon deposit that penetrated into the interior of the catalyst particles. An optimum temperature around 227°C. was found for the

TABLE 2

Alkylation of isobutane with propene over lithium aluminum chloride on pumice
(Blunck and Carmody)

Run No.....	42-A	42-B	43-A	43-B
Temperature, °C.....	285	287	288	291
Pressure, psi.....	1000	1200	1200	1200
Flow rate (liquid feed volume per volume of catalyst per hour).....	1.59	1.59	1.76	1.57
Duration, hours.....	5.0	4.5	6	8
Feed composition, mole per cent:				
Isobutane.....	67	65	65	65
Propene.....	33	35	35	35
Catalyst, cc.....	100	100	100	100
Yield, per cent*.....	17.0	9.8	17.8	11.0
Products, liquid volume per cent on C_{5+} :				
C_5	6.6	0.6	2.5	1.3
Unsaturated in C_5	1.5			
C_6	11.6	6.5	10.3	6.0
Unsaturated in C_6	1.5	28.2	9.5	48.0
C_7	35.6	16.7	25.8	11.2
Unsaturated in C_7	2.5	40.3	14.0	65.7
C_8	11.1	15.8	12.9	13.3
Unsaturated in C_8	6.5	52.0	18.5	67.2
C_9	12.8	21.0	14.3	24.3
Unsaturated in C_9	35.7	9.0	58.7	25.0
C_{10+}	22.3	39.4	33.2	43.9

* Weight per cent yield, based on the weight of heptane that would have been produced by the union of all the alkene in the feed, mole for mole, with isobutane.

production of liquid hydrocarbons from a feed containing 35–40 mole per cent of propene in isobutane over lithium aluminum chloride on pumice and 1000–1200 psi (figure 2, top curve). The lower portion of figure 2 gives the volume percentage of individual hydrocarbon cuts of the product, measured by the distance between solid curves, and the volume percentage of alkenes in these cuts as the distance between a broken line and the solid line immediately below it. A rise in temperature increased the catalyst's selectivity towards alkylation into heptanes at the expense of the polymer products, total yield of liquid products, and life of the catalyst.

Table 3 gives the results for the alkylation of isobutane by ethene, propene,

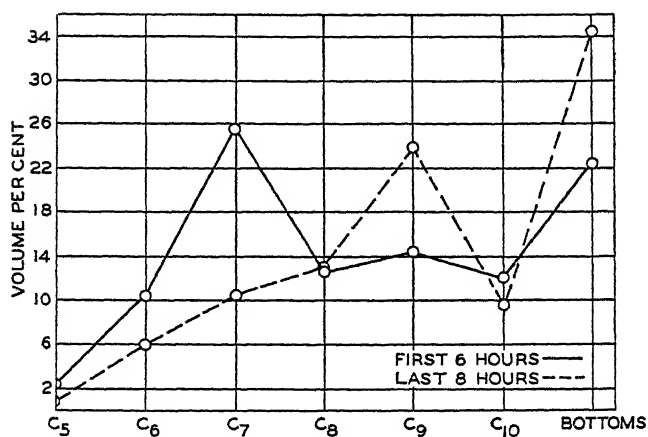
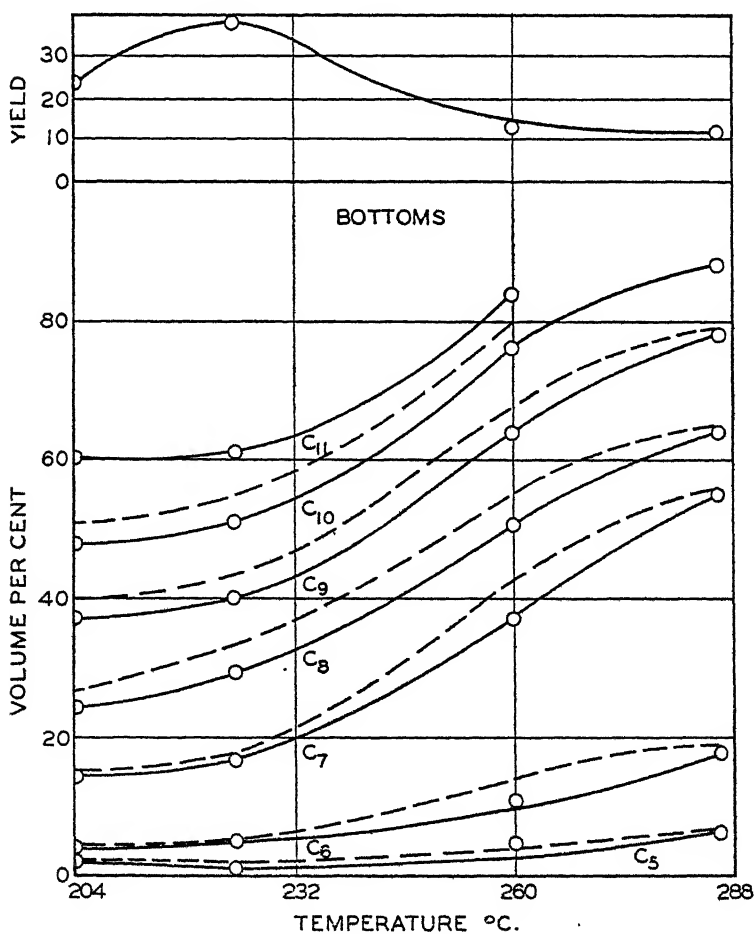


FIG. 1. Effect of age of catalyst on distribution of products (Blunk and Carmody)



Run No.	ISOPUTENE						PROPENE			ETHENE		
	1	2	3	4	5	6	7	8	9	10	11	12
Feed, mole per cent:												
Isobutane.....	95	90	90	85	77	90	80	80	50	95	94	85
Alkene.....	5	10	10	15	23	10	20	20	50	5	6	15
Temperature, °C.....	204	204	157	204	171	196	104	218	218	154	171	171
Pressure, psi.....	1000	1000	1000	1000	1000	0	1000	1000	1000	1000	1000	1000
Flow rate, vol./vol./hr.....	1.5	2.1	4.8	1.6	5.0	1.5	3.7	3.6	3.8	4.0	3.2	1.8
Duration of run, hours.....	2.5	3.25	2.5	4.5	2.0	3.0	2.2	2.0	2.4	2.0	2.0	3.0
Yield, per cent*.....	93	83	27		60	32.5	46	56	25	30	25	
Products, liquid volume per cent on C ₄₊ :												
C ₄ , per cent.....	14.8	16.8	8.8	17.6	3.0	11.8	13.0	11.8	12.0	6.2	13.0	12.5
Unsaturated, per cent.....	4.0	4.0	8.0	10.0	30.0	35.0	Slight†	Slight	5	Complete saturation	Complete saturation	Complete saturation
n _D ²⁰	1.3695	1.3560	1.3562	1.3560	1.3583	1.3745	1.3560	1.3615	1.3615	1.3620	1.3615	1.3624
C ₃ , per cent.....	9.6	9.6	7.2	7.7	5.5	8.8	9.3	8.5	10.0	12.4	29.0	25.0
Unsaturated, per cent.....	10	10	10	16	35	40	0	Slight	7	Complete saturation	Complete saturation	Complete saturation
n _D ²⁰	1.3810	1.3765	1.3769	1.3782	1.3710	1.3845	1.3746	1.3740	1.3754	1.3680	1.3695	1.3699
C ₂ , per cent.....	6.8	9.3	5.6	7.7	5.1	5.9	17.1	15.8	12.0	12.4	13.0	12.5
Unsaturated, per cent.....	6.0	14.0	9.0	16.0	25	35	0	Slight	8	Complete saturation	Complete saturation	Complete saturation
n _D ²⁰	1.3960	1.3920	1.3860	1.3920	1.3920	1.4055	1.3850	1.3879	1.3865	1.3810	1.3850	1.3844
C ₁ , per cent.....	29.5	30.0	40.8	29.5	38.0	44.1	14.5	11.0	11.0	37.2	32.0	25
Unsaturated, per cent.....	14.1	13.0	10.0	13.4	20.0	30.4	0	Slight	8	Complete saturation	Complete saturation	Complete saturation
n _D ²⁰	1.4090	1.4060	1.3900	1.4075	1.4110	1.4170	1.3890	1.3879	1.3990	1.3915	1.3916	1.3917
C ₄₊ (bottoms).....	49.3	44.0	37.0	37.0	48.4	29	46.1	52.9	55.0	31.8	13.0	25.0

* For definition, see table 2.

† Within the experimental error of 2 per cent.

or isobutene over sodium aluminum chloride on pumice. The optimum temperature for this catalyst was stated to be in the neighborhood of 218°C. In the case of ethene, the C₅, C₆, C₇, and C₈ fractions of the product were completely saturated. This indicates a tendency to alkylate instead of polymerize. Isobutene, however, led to corresponding fractions with 4-40 per cent of alkenes. Propene gave fractions with intermediary percentages of unsaturates. Alkylation was progressively easier in the order ethene, propene, isobutene, but the specificity of the reaction decreased; this indicates that the polymerization tendency increased more rapidly than the alkylation tendency. Figure 3 gives the effect of increased space velocity of feed containing 50 mole per cent each of iso-

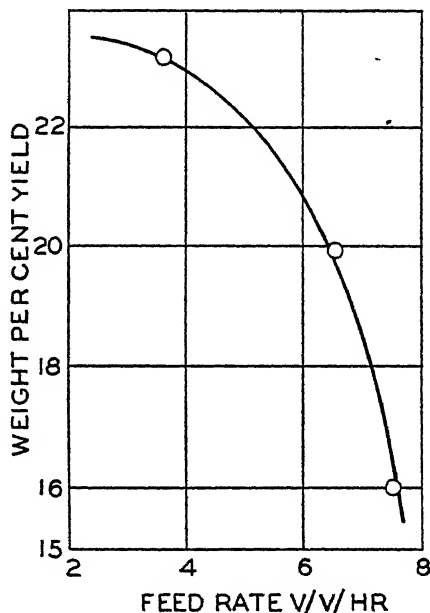


FIG. 3. The effect of increased space velocity of feed containing 50 mole per cent each of isobutane and propene upon the weight per cent yield of liquid products at 218°C. and 1000 psi over sodium aluminum chloride on pumice (Blunck and Carmody).

butane and propene upon the weight per cent yield of liquid products at 218°C. and 1000 psi over sodium aluminum chloride on pumice. A feed rate greater than 4.0 volumes of feed per volume of catalyst per hour caused the yield to fall off rapidly. The corresponding products were more unsaturated, in agreement with a considerably slower rate for alkylation than for polymerization.

C. ALUMINUM BROMIDE

Alkylations of *n*-butane and isobutane by methyl or ethyl bromide have been reported (23). The treatment of *n*-butane with methyl bromide and aluminum bromide at 25-78°C. gave isobutane and isopentane to the extent of 20.6 and 1.5-17.3 mole per cent on *n*-butane introduced, respectively. No alkylation or

isomerization of *n*-butane occurred in the presence of methyl chloride and aluminum chloride at 25°C. This inactivity was probably due to complete removal of aluminum chloride as a soluble complex (isolated) containing methyl chloride. *n*-Butane, ethyl bromide, and aluminum bromide at 25°C. yielded isobutane and an alkylate containing hexanes, corresponding to 47.7 and 20.8–30 mole per cent

TABLE 4
Alkylation of alkanes by alkyl halides
(Heldman*)

REACTANTS						CONDITIONS		PRODUCTS†	
Alkane		Alkyl halide		Catalyst		Time	Temperature	Formula	Moles
Formula	Moles	Formula	Moles	Formula	Moles	hours	°C.		
C ₂ H ₆	0.0230	CH ₃ Br	0.0392	Al ₂ Br ₆	0.00167	120 ≈	25 ± 3		0
<i>n</i> -C ₄ H ₁₀	0.0500	CH ₃ Br	0.0392	Al ₂ Br ₆	0.00143	95.8	25.0	Iso-C ₄ H ₁₀	0.0103
								Alkylate	<0.00075
<i>n</i> -C ₄ H ₁₀	0.0750	CH ₃ Br	0.0392	Al ₂ Br ₆	0.00158	120	25 ± 3	Iso-C ₅ H ₁₂	0.0052
<i>n</i> -C ₄ H ₁₀	0.0250	CH ₃ Br	0.0392	Al ₂ Br ₆	0.00181	96	25.0	Alkylate	0.0033
<i>n</i> -C ₄ H ₁₀	0.0500	CH ₃ Br	0.0392	Al ₂ Br ₆	0.00342	68.8	25 ± 3	Alkylate	0.0083
<i>n</i> -C ₄ H ₁₀	0.0753	CH ₃ Br	0.0390	Al ₂ Br ₆	0.00183	64.8	78 ± 2	Iso-C ₅ H ₁₂	0.0130
<i>n</i> -C ₄ H ₁₀	0.0500	CH ₃ Cl	0.0860	Al ₂ Cl ₆	0.00292	90	25 ± 3		0
<i>n</i> -C ₄ H ₁₀	0.0250	C ₂ H ₅ Br	0.0183	Al ₂ Br ₆	0.000638	72	25.0	Iso-C ₄ H ₁₀	0.0119
								Alkylate	0.0052
<i>n</i> -C ₄ H ₁₀	0.0500	C ₂ H ₅ Br	0.0262	Al ₂ Br ₆	0.00252	42	25 ± 3	Alkylate	0.0150
Iso-C ₄ H ₁₀	0.0520	CH ₃ Br	0.0392	Al ₂ Br ₆	0.00142	283	25 ± 3	<i>n</i> -C ₄ H ₁₀	0.0062
								Iso-C ₄ H ₁₀	0.0410
								Iso-C ₅ H ₁₂	0.0034
								Higher alkylate	0.0024
Iso-C ₄ H ₁₀	0.0494	CH ₃ Br	0.0580	Al ₂ Br ₆	0.00169	47.8	50 ± 1	Alkylate	0.0062
Iso-C ₄ H ₁₀	0.0741	C ₂ H ₅ Br	0.0642	Al ₂ Br ₆	0.00310	47.8	50 ± 1	CH ₄	trace
								C ₂ H ₆	0.0140
								C ₃ H ₈	0.0080
								<i>n</i> -C ₄ H ₁₀	0.0082
								Iso-C ₄ H ₁₀	0.0185
								Iso-C ₅ H ₁₂	0.0130
								Higher alkylate	0.0095

* Heldman's original data retabulated by the present authors.

† Including unconverted or isomerized starting alkane and lower alkanes when reported.

on *n*-butane introduced, respectively. Isobutane, methyl bromide, and aluminum bromide at 25°C. formed *n*-butane, isopentane, and higher alkanes, amounting respectively to 12, 6.5, and 4.6 mole per cent on isobutane introduced. With isobutane, ethyl bromide, and aluminum bromide at 50°C., the products were ethane, propane, *n*-butane, isopentane, and higher alkanes including hexane, corresponding respectively to 18.9, 10.8, 11.1, 17.5, and 12.8 mole per cent on isobutane introduced. No alkylation of propane by methyl bromide and

aluminum bromide at 25°C. occurred even with a contact time of 120 hr. These alkylation experiments are summarized in table 4. Large quantities of lower alkanes are indicated as by-products of the reaction of isobutane with ethyl bromide at 50°C. The possibility of an autodestructive alkylation of butanes



should not be forgotten in an evaluation of the foregoing experiments.

D. ALUMINUM BROMIDE AND ALUMINUM CHLORIDE

n-Butane has been alkylated by ethene under 15 atmospheres pressure in the presence of a mixture of aluminum bromide and aluminum chloride without any hydrogen halide as a promoter (24b). About 44.5 per cent of the *n*-butane enters into the reaction. A typical product is obtained, the upper layer of which contains alkanes distilling from 45°C. to above 150°C. The lower layer yields a small amount of unsaturated hydrocarbons when treated with ice water. We presume that aluminum bromide is the more active (less associated) component of the mixed catalyst.

E. ZIRCONIUM CHLORIDE

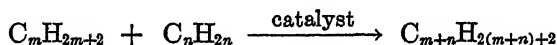
Ipatieff and his coworkers have disclosed that zirconium chloride is a considerably less active catalyst than aluminum chloride in the alkylation of isobutane by ethene; a temperature of 100°C. or over is required (25). About 50 per cent of isobutane was alkylated by ethene under 15 atmospheres pressure at 100°C. in the presence of zirconium chloride. The product consisted of a colorless mobile liquid and a dark pasty mass exhibiting catalytic activity. In the colorless liquid were alkanes distilling up to 200°C. and representing 70 per cent of all hydrocarbon produced. These alkanes were formed by the addition of one to three molecules of ethene to a molecule of isobutane. Practically all of the zirconium chloride was contained in the dark pasty mass. This was used as catalyst for a 34-hr. run in which isobutane was alkylated by ethene at 100°C. and 35-45 atmospheres pressure.

F. BORON FLUORIDE

Alkylation of isoalkanes with alkenes has been carried out in the presence of boron trifluoride, finely divided nickel, and small quantities of water or hydrogen fluoride (28). The reaction took place at room temperature, although temperatures as high as 200°C. were used occasionally, and a pressure of 5-20 atmospheres was found desirable. Alkylations of (1) isobutane with ethene, (2) isopentane with ethene, (3) 2,2,4-trimethylpentane with ethene, and (4) isobutane with isobutene were reported (table 5).

The data indicate that the alkylations were complicated by side reactions producing alkanes with an intermediary number of carbon atoms. From the wide range of products formed, it is obvious that the ethylation or butylation of lower isoalkanes was not highly selective, although alkylation without cracking was about 75 per cent. The general reaction was considered to be a direct addi-

tion of an alkene to the tertiary carbon atom of an isoalkane, according to the equation:



A further alkylation gives alkanes with the formula $C_{m+2n}H_{2(m+2n)+2}$. An abnormal behavior of 2,2,4-trimethylpentane under ethylation was observed; the isoalkane reacted slowly, and the ethene polymerized in part to high-boiling unsaturated hydrocarbons.

A later paper (20) describes the products from the alkylation of isobutane by ethene at 0–5°C. or –30° to –40°C. in the presence of boron fluoride, hydrogen fluoride, and nickel. In the experiment at 0–5°C., hexanes made up 45 per cent

TABLE 5

Alkylation of isoalkanes by alkenes in the presence of boron fluoride, hydrogen fluoride, and nickel

(Compiled from data of Ipatieff and Grosse)

Isoalkane used.....	Isobutane	Isopentane	2,2,4-Trimethyl- pentane	Isobutane
Alkene used.....	Ethene	Ethene	Ethene	Isobutene
Temperature, °C.....	20–30	150	25	25
Composition of the reaction product (volume per cent):				
Isobutane.....	7	0		11
Isopentane.....	2	60		5.2
Isohexanes.....	19	6.6		
Isoheptanes.....	6	5.8		2.7
Isooctanes.....	22	5.9	+	32
Isononanes.....	44	3.8		12.6
Isodecanes.....		3.3	5–10	
Isoundecanes.....				
Isododecanes.....		14.6	+	15
Higher alkanes.....				21.5

of the total liquid product. The hexane fraction in all cases contained 10–25 per cent of 2-methylpentane, less than 3 per cent of 2,2-dimethylbutane, and 70–90 per cent of 2,3-dimethylbutane. Other hexanes were probably absent; the products were investigated both chemically and Raman spectroscopically (21). The possibilities of side reaction were also considered (20):

“It should be stressed that in catalytic reactions the lowering of the energy barrier of the desired reaction brought about by the particular catalytic mechanism, markedly increases also the probability of undesired reactions. This is particularly true for reactions among hydrocarbons. Furthermore, the energy given off by the desired reaction stimulates the undesired reactions by providing the necessary activation energy. The cumulative effect is such that it is highly optimistic to expect the simple reaction mixture encountered in most inorganic or organic reactions. In the field of catalytic hydrocarbon reactions one can hope to surmount these difficulties only by picking the simplest hydrocarbons or de-

veloping highly selective catalysts and allowing them to work under carefully selected conditions."

Such catalysts and conditions have since been found.

G. HYDROGEN FLUORIDE

1. Alkenes

Alkylations of isobutane and isopentane have been effected by propene or butenes in the presence of liquid hydrogen fluoride (35). Owing to wartime

TABLE 6
Alkylation of isobutane and isopentane
(Linn and Grosse)

Temperature, °C.....	10	10	10	10	10	10	10	38
Reaction time, hours...	5.0	5.0	5.0	5.0	5.0	5.0	5.0	
Catalyst:hydrocarbon ratio.....	0.13	0.16	0.16	0.16	0.16	0.15	0.15	1.1
Isoalkane:alkene mole ratio.....	3.5	3.0	3.3	3.2	3.5	3.3	1.5	6
Charging stock, mole per cent:								
Propene.....	22	25	0	0	0	0	0	4‡
Isobutene.....	0	0	0	0	22	19	20	
1-Butene.....	0	0	21	4	0	0	0	2
2-Butene.....	0	0	2	20	0	0	0	9.5
Isobutane.....	0	75	77	76	78	62	30	72
n-Butane.....	0	0	0	0	0	19	50	11.5
Isopentane.....	78	0	0	0	0	0	0	0.5
Liquid alkylate yield:								
Weight per cent alkene reacted..	272*	230	194	203	197	210	168	227
Fluorine in alkylate, per cent†..	0.08	0.01	0.01	0.01	0.01	0.01	0.01	0.026
150°E.P. gasoline, weight per cent of product	73	81	85	91	91	89	69	95
Octane No., 150°E.P. gasoline...		90.5	92.7	95.3	96.7	95.6	92	94.9

* Exclusive of pentanes; counting isobutane formed as product, the yield was 291 per cent.

† Not treated to remove fluorine. In commercial operations this is always done. After treatment the fluorine content was 0.0056 per cent.

‡ Propene + propane.

restrictions considerable data remain unpublished, though several patents have been granted. Some of the accessible data are summarized in tables 6-8.

Table 6 contains data for seven batch runs carried out with pure reactants at 10°C. and one continuous run using a commercial butane-butene cut (BB fraction) at 38°C. The interaction of isobutane and propene gave a desirable product containing 50 per cent of heptanes, including 2,3-dimethylpentane, besides 2,2,4-trimethylpentane. 2-Butene showed a definite advantage over 1-butene in the alkylation of isobutane to form a motor fuel. 2-Pentene likewise showed similar advantages over 1-pentene in the alkylation of isobutane. The products

from these 2-alkene examples were superior in octane number and aviation gasoline content. Isobutane and isobutene formed much 2,2,4-trimethylpentane, which was reflected in the 96.7 octane number of the 150°C. end point gasoline produced. A marked reduction in the yield and quality of the alkylate occurred when the charge contained 50 per cent of *n*-butane. Isopentane and propene reacted non-selectively, producing 291 weight per cent of alkylate based on propene reacted. Isobutane and only 26 per cent of octanes were present in the product, indicating that an autodestructive alkylation had occurred. In all cases the fluorine content of the alkylate was low and easily removed by activated charcoal, calcium fluoride, or aluminum fluoride at elevated temperatures.

Table 7 identifies some of the alkanes formed in the alkylation of isobutane and isopentane. Isobutane and propene produced 2,3- and 2,4-dimethylpentanes, which products were formed along with 2,3- and 2,5-dimethylhexanes from isopentane and propene. Both *n*-butenes and isobutene reacted with isobutane to form 2,2,4-trimethylpentane.

TABLE 7
Alkanes identified in reaction products
(Linn and Grosse)

ALKANES USED	ALKENES USED	BOILING RANGE OF SAMPLE AT 750 MM. °C.	ALKANES IDENTIFIED
Iso-C ₄ H ₁₀	C ₃ H ₆	75.7- 79.0	2,4-Dimethylpentane
Iso-C ₄ H ₁₀	C ₃ H ₆	83.5- 85.5	2,4-Dimethylpentane
Iso-C ₄ H ₁₀	<i>n</i> -C ₄ H ₈	98.0- 99.0	2,3-Dimethylpentane
Iso-C ₄ H ₁₀	Iso-C ₄ H ₈	98.0- 99.0	2,2,4-Trimethylpentane
Iso-C ₅ H ₁₂	C ₃ H ₆	79.5- 81.0	2,4-Dimethylpentane
Iso-C ₅ H ₁₂	C ₃ H ₆	89.0- 90.0	2,3-Dimethylpentane
Iso-C ₅ H ₁₂	C ₃ H ₆	108.0-110	2,5-Dimethylhexane
Iso-C ₅ H ₁₂	C ₃ H ₆	115.0-116.0	2,3-Dimethylhexane

Table 8 gives data on the alkylation of isobutane by isobutene over an 85°C. temperature range. It indicates that the temperature can be varied considerably without adverse effect upon the properties of the alkylate.

The principal reactions, other than physical dissolution of hydrocarbons, occurring in the reaction zone under optimum conditions are apparently as follows:

- (1) Direct interaction of an isoalkane and alkene, either of which may be charged as such or may be the result of previous reactions.
- (2) Dealkylation of alkanes of high molecular weight.
- (3) Polymerization of alkenes to form alkenes of higher molecular weight.
- (4) Depolymerization.
- (5) Hydrogen-transfer reactions between alkenes to form saturated hydrocarbons and tars of low hydrogen content.
- (6) Hydrogen-transfer reactions between alkanes and alkenes to form an alkane corresponding to the alkene and an alkene corresponding to the alkane.

(7) Chain isomerization, particularly of the alkylate product.

A number of reactions take place, and a variety of products may be made by changing the balance of these reactions. The most desirable products are those of the direct combination of the isoalkane and alkene feeds. Conditions should be so chosen as to favor such a reaction within the limits of commercial practicability. The means of adjusting the balance between reactions lies in the control of the concentrations of the various reactants and reaction products. Isobutane alkylations are adversely affected by the presence of water in hydrogen fluoride; an excess of water leads to formation of alkyl fluorides.

Typical Raman spectrum analyses (21) of various narrow-boiling fractions obtained in the alkylation of isobutane and isopentane by alkenes in the presence of hydrogen fluoride or of other catalysts yielding similar alkylate fractions are given in table 9.

TABLE 8
Isobutane-isobutene alkylation
(Linn and Grosse)

Temperature, °C.....	-24	32	60
Hydrocarbon:HF ratio, by volume.....	0.8	0.2	0.8
Charge, grams:			
Iso-C ₄ H ₈	177	344	168
Iso-C ₄ H ₁₀	420	561	397
Butane-free product, grams.....	351	670	298
Properties of product:			
Bromine number.....	0	0	0
Per cent fluorine.....	0.4	0.1	0.6
Engler 50% Point, °C.....	127	115	130
Engler 205°C., E.P., per cent.....	85	94	83
Octane number, A.S.T.M. motor method.....	91.5	92.0	89.0

Alkylation of isobutane by propene, butenes, or pentenes in the presence of anhydrous liquid hydrogen fluoride has been developed to successful commercialization (19). Figure 4 is a schematic drawing of a typical alkylation unit. Single units containing as much as 20,000 gallons of hydrogen fluoride are in operation for the production of aviation gasoline with about 93 A.S.T.M. octane number. Charging stock to the units passes through charge pretreaters that remove organic impurities and water. Recycle isobutane is fed directly and in abundance to the reaction system, ensuring satisfactory alkylation. The reaction is exothermic and is carried out advantageously at higher process temperatures than those used in alkylation with sulfuric acid. Defluorinating agents such as bauxite are used to remove from the final product small amounts of alkyl fluorides, which are converted into alkenes and hydrogen fluoride.

Operating features of the first commercial hydrogen fluoride alkylation plant were revealed after a year of operation by the Phillips Petroleum Company

(17). Bauxite driers have been used to remove small amounts of water dissolved in isobutane and the alkene-containing feed (usually butenes but can be propene, pentenes, or higher alkenes). Hydrocarbon streams have been proportioned to give a desired several-fold excess of isobutane over alkene and then

TABLE 9
Data on alkylation
(Grosse, Rosenbaum, and Jacobson)

BOILING RANGE OF SAMPLE AT 750 mm.	ISOALKANE	ALKENE	CATALYST	TEMPERATURE OF REACTION	ISOALKANES IDENTIFIED
°C.				°C.	
56.0- 57.0	Iso-C ₄ H ₁₀	C ₂ H ₄	AlCl ₃ , HCl	+30	100% 2,3-dimethylbutane
59 - 60	Iso-C ₄ H ₁₀	C ₂ H ₄	BF ₃ , H ₂ O	+30	90% 2,3-dimethylbutane; 10% 2-methylpentane
59 - 60.5	Iso-C ₄ H ₁₀	C ₂ H ₄	Solid H ₃ PO ₄	400	2-Methylpentane
79.0- 81.0	Iso-C ₄ H ₁₀	C ₃ H ₆	BF ₃ , HF	0	100% 2,4-dimethylpentane
85.0- 86.3	Iso-C ₄ H ₁₀	C ₃ H ₆	BF ₃ , HF	0	100% 2,3-dimethylpentane
95.0- 97.0	Iso-C ₄ H ₁₀	C ₃ H ₆	BF ₃ , HF	0	100% 2,2,4-trimethylpentane
75.7- 79.0	Iso-C ₄ H ₁₀	C ₃ H ₆	HF, 100%	+30	100% 2,4-dimethylpentane
83.5- 85.5	Iso-C ₄ H ₁₀	C ₃ H ₆	HF, 100%	+30	45% 2,4-dimethylpentane; 55% 2,3-dimethylpentane
83.5- 85.5	Iso-C ₄ H ₁₀	C ₃ H ₆	AlCl ₃ , HCl	-30	50% 2,4-dimethylpentane; 50% 2,3-dimethylpentane
108.5-110.0	Iso-C ₄ H ₁₀	n-C ₄ H ₈	AlCl ₃ , HCl	-30	65% 2,5-dimethylhexane; 15% 2,2,3-trimethylpentane; 5% 2,2,4-trimethylpentane
113.0-114.0	Iso-C ₄ H ₁₀	n-C ₄ H ₈	AlCl ₃ , HCl	-30	Mainly 2,3,4-trimethylpentane
98.0- 99.0	Iso-C ₄ H ₁₀	n-C ₄ H ₈	HF, 100%	+30	100% 2,2,4-trimethylpentane
98.0- 99.0	Iso-C ₄ H ₁₀	Iso-C ₄ H ₈	HF, 100%	+30	100% 2,2,4-trimethylpentane
79.5- 81.0	Iso-C ₅ H ₁₂	C ₃ H ₆	HF, 100%	+30	95% 2,4-dimethylpentane; 5% 2,2,3-trimethylbutane
89.0- 90.0	Iso-C ₅ H ₁₂	C ₃ H ₆	HF, 100%	+30	100% 2,3-dimethylpentane
108.0-110	Iso-C ₅ H ₁₂	C ₃ H ₆	HF, 100%	+30	Nearly pure 2,5-dimethylhexane
115.0-116.0	Iso-C ₅ H ₁₂	C ₃ H ₆	HF, 100%	+30	100% 2,3-dimethylhexane
89.0- 90.0	Iso-C ₅ H ₁₂	n-C ₄ H ₈	H ₂ SO ₄ , 98%	+30	60% 2,3-dimethylpentane; 40% 2-methylhexane
58.0- 60.0	Iso-C ₅ H ₁₂	n-C ₄ H ₈	H ₂ SO ₄ , 98%	+30	50% 2,3-dimethylbutane; 50% 2-methylpentane

introduced into reactors where the mixed hydrocarbons have come in contact with about an equal volume of recycle acid for periods of less than an hour at temperatures between 24° and 46°C. The exothermic heat of alkylation has been removed by cooling coils in the reactors. Recycle acid has been partially

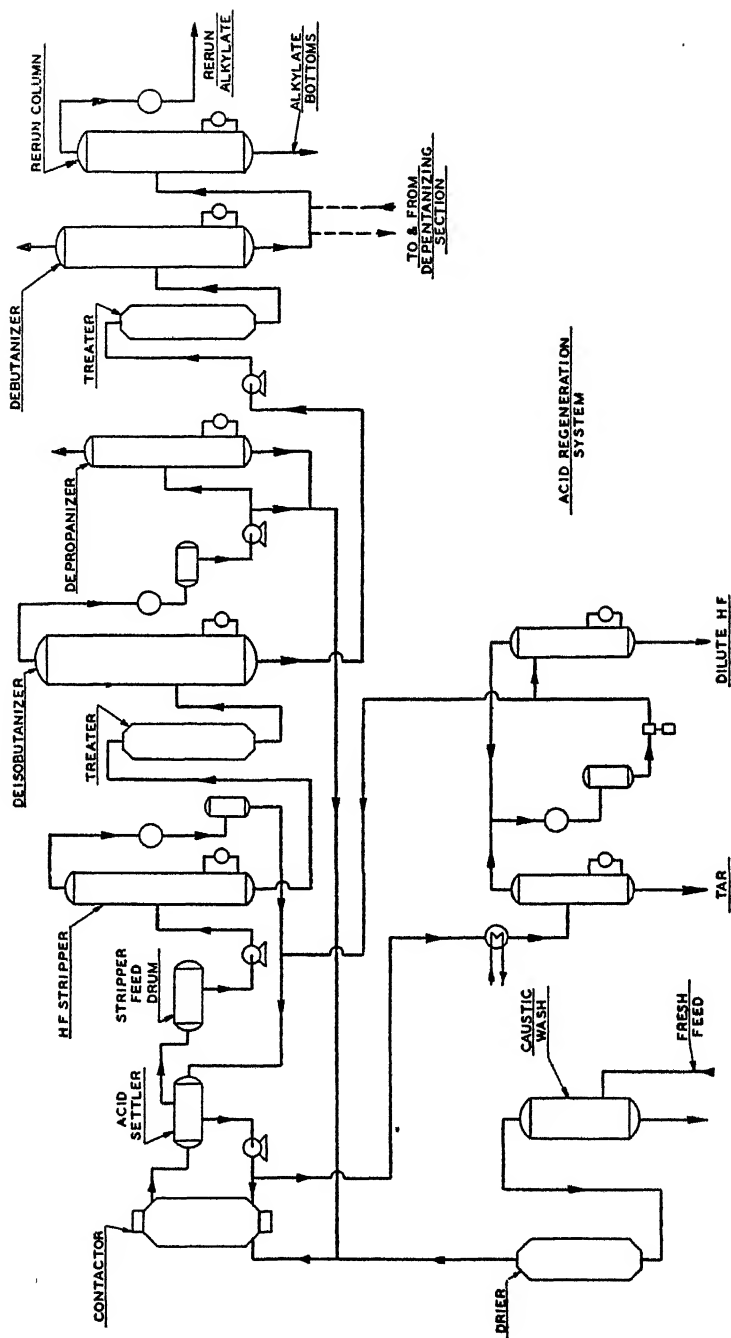


Fig. 4. Typical hydrogen fluoride alkylation unit

purified of high-boiling organic contaminants. The few per cent of such organic compounds remaining in the recycle acid have minimized the decomposition of primary alkylate; an excess of organic contaminants in the recycle acid would cause a production of organic fluorides instead of hydrocarbon products. A titratable acidity of 85–90 per cent has been usually maintained in the catalyst phase. The consumption of hydrogen fluoride in the form of physical and chemical losses has been kept at a rather low value.

2. Alkyl fluorides

Alkylation of isoalkanes by alkyl fluorides in the presence of anhydrous hydrogen fluoride (34) gives products similar to those obtained from the interaction of isoalkanes, alkenes, and hydrogen fluoride. The reaction is less exothermic and the products contain only traces of organic fluorides. Table 10 gives the ex-

TABLE 10
Alkylation of isoalkanes with alkyl fluorides in presence of hydrogen fluoride
(Linn)

Reactants used, grams:			
Isobutane..	331	335	0
Isopentane	0	0	112
Isopropyl fluoride	106	0	79
sec-Butyl fluoride.	0	121	0
Temperature of experiment, °C.. . . .	37	10	35
Liquid alkanes recovered, grams:			
C ₅ and higher	163	184	
C ₆ and higher			132
Weight per cent fluorine.....	0.01	0.015	0.1
Composition of product, weight per cent:			
C ₅ alkanes.....	6	5.9	
C ₆ alkanes.	7	4.3	12
C ₇ alkanes..	41	3.6	10
C ₈ alkanes	29	67.8	23
C ₉ alkanes	4	18.4	13
Higher alkanes	13		42

perimental results for batch operation, using isobutane with isopropyl fluoride or sec-butyl fluoride and isopentane with isopropyl fluoride. No significant difference in composition of products from the interaction of isobutane with sec-butyl fluoride or 2-butene was found by infrared analysis.

H. SULFURIC ACID

No alkylation of an alkane or isoalkane by ethene in the presence of sulfuric acid has been reported. One group of investigators writes (54): "It was observed that ethylene and the normal paraffins are not appropriate materials for the condensation reaction at atmospheric pressure with sulfuric acid as a catalyst (in these series of experiments 98% sulfuric acid was always used)." This may be due to stability of normal alkanes, ethyl hydrogen sulfate, and diethyl sulfate at the usual alkylation temperatures.

TABLE II
Diisobutene and isobutane at 20°C., using 96.9 per cent acid
 Time of addition, 90 min.; time of stirring, 30 min.

RUN NO.	ISOBUTANE: DIISOBUTENE RATIO	PRODUCT >27°C.										PRODUCT 27-185°C.						RESIDUE									
		A. S. T. M. Distillation										Octane rating						Octane rating									
		On alkene taken										15.6°C.						+1.5 cc. T. B. L. per (Imperial) gallon									
		I.B.P.	2%	5%	10%	20%	30%	40%	50%	60%	70%	80%	90%	F.B.P.	T.D.	Residue	On alkene taken	Bromine number†	Specific gravity at 15.6°C.	50% in H.O.S.‡	Unleaded	On alkene taken	Bromine number†	Specific gravity at 15.6°C.			
42A	1:1	165	38	45.5	63	594	105	112.5	117.5	125.5	137.5	160.5	197	257	97.5	1.5	136	<10	705	71.2	90.5	98	22	14	0.783		
51*	1:1	167	35.5	63.5	78.5	98.5	108	114.5	120.5	128.5	140	165	200	264.5	97.5	1.5	142	<10	701	71.6	90.8	99.2	19.6	10	0.782		
55	2:1	184	33	39	49	64	89	101	107	112	118	128	148	194	230	96	51	158	<10	700	71.1	91.3	98	9	14.8	9.5	0.782
59	4:1	190†	36.5	55	68	81	96.5	103	107	110.5	114.5	120	133.5	172	220	97.5	1.5	167¶	<10	705	72.3	92.2	101	10.8	10	0.777	

* Repeat run with improved stirrer.

† An engine test on the product boiling over 27°C. (i.e., including the residue) gave 90.6 octane number and 1.5 cc. T.E.L. per Imperial gallon 100 octane number.

‡ Francis method.

§ High-octane standard. A straight-run Iranian cut of 52.5 octane number.

¶ Sulfur, 0.01 per cent.

Birch, Dunstan, Fidler, Pim, and Tait were the first to publish a study on the alkylation of isobutane, isopentane, and 2-methylpentane by alkenes at 20°C. in the presence of 97 per cent sulfuric acid, emphasizing the isoalkane character of the products boiling from 27° to 185°C. and also their value as high-octane-rating motor fuels (9). Propene, however, reacted only when used with sulfuric acid of 100.6 per cent concentration, forming (at 30°C.) an 82.5-octane-rating fuel and a residue with 2.5 bromine number (Francis). Isobutane did not react with 1-butene as readily as with 2-butene, though the quality and quantity of hydrocarbons formed were comparable. With 2-butene it gave a considerable quantity of 2,2,4-trimethylpentane. Isobutene and its polymers (diisobutene and triisobutene) were observed to give "identical products" in the alkylation of isobutane. Apparently a depolymerization of the polyisobutenes occurs under

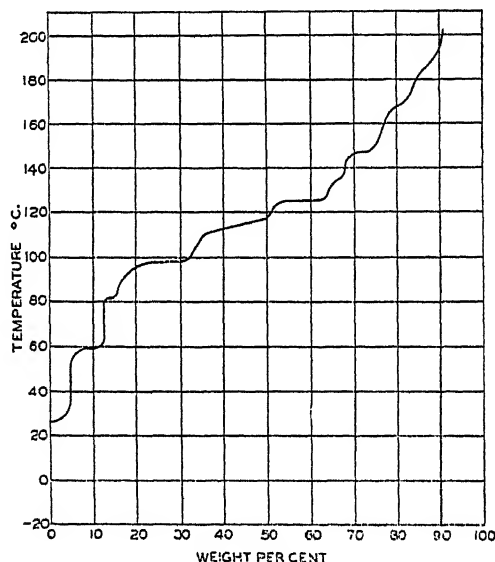


FIG. 5. Distillation curve (25-plate column) of product from diisobutene and isobutane at 20°C. $(C_4H_8)_2:C_4H_{10} = 1:2$. Run 59. (Birch, Dunstan, Fidler, Pim, and Tait)

reaction conditions. (We shall enclose the word "isobutene" in quotation marks whenever, owing to interchangeability of isobutene and its polymers in alkylation, the source of isobutene is considered to be inadequately disclosed in the literature.) The common product was found to contain isopentane, 2,3-dimethylbutane, 2,3- and 2,4-dimethylpentanes, 2,5-dimethylhexane, 2,2,4-trimethylpentane, and 2,2,5-trimethylhexane. An excess of isobutane over that required for equimolecular proportions of isobutane to "isobutene" led to an increased yield of motor fuel at the expense of high-boiling residue. Such motor fuel had an increased content of isoöctanes and showed a definite improvement in octane rating (table 11 and figure 5). The product from isobutane and "isobutene" in 4:1 ratio had a final boiling point of 220°C. and 90.6 octane rating; addition of 1.5 cc. of tetraethyllead per Imperial gallon gave a fuel with 100.0 octane number.

Other tests with isobutane and "isobutene" at -10°C . showed a drop in motor fuel yield, a slight improvement in octane rating, and the formation of a considerable amount of high-boiling residue. At temperatures above 20°C . an oxidation developed at the expense of sulfuric acid, which was "fouled" and reduced to sulfur dioxide. The hydrocarbon product showed a slightly decreased octane rating. Consequently, the optimum temperature point was considered to be 20°C .

Isobutane was also alkylated by refinery unsaturated C_4 fractions, 2-methyl-2-butene, butene-isobutene copolymers of $95\text{--}120^{\circ}\text{C}$. or $105.5\text{--}112^{\circ}\text{C}$. boiling range, and polymer gasoline ($80\text{--}120^{\circ}\text{C}$. boiling range; prepared from C_3 and C_4 alkenes). Refinery unsaturated cuts, consisting of all C_4 alkanes-alkenes and some butadiene, yielded a high-octane-rating motor fuel when reacted with an excess of isobutane. The polymer of $105.5\text{--}112^{\circ}\text{C}$. boiling range was found to alkylate isobutane at 20°C . in the presence of 97 per cent sulfuric acid, though the yield and octane rating of the motor fuel fraction were slightly low. Satisfactory results were obtained with polymer of $95\text{--}120^{\circ}\text{C}$. boiling range; the product closely resembled those from isobutane and polyisobutene, from isobutane and 1-butene, or from isobutane and 2-butene. Alkylations of isopentane and 2-methylpentane were similarly effected at 20°C . by contact with diisobutene and 97 per cent sulfuric acid. Table 12 summarizes most of the foregoing experiments. The product yields are based upon the weight of alkene taken. Thus the theoretical yield for the alkylation of isobutane by diisobutene is 204 per cent. Isooctane (2,2,4-trimethylpentane), which contains a tertiary carbon atom, failed to undergo alkylation.

Alkylation of isopentane by means of 2-methyl-2-butene and 98 per cent sulfuric acid has been investigated (54). A small amount of 2-methyl-1-butene was probably present in the 2-methyl-2-butene. The investigators dropped a mixture of the isoalkane and alkene into sulfuric acid maintained at $0\text{--}9^{\circ}\text{C}$. Two experiments were conducted, using 3:1 and 1:1 volume ratios of isoalkane and alkene and reaction times of 22–40 min.

With the 3:1 ratio of isopentane to 2-methyl-2-butene, the product consisted essentially of isoalkanes, including isobutane, an isohexane, an isononane, and the expected decane fraction. The high-boiling fractions were slightly unsaturated and probably non-cyclic in structure. A very good yield of products with a molecular weight higher than that of the raw materials was obtained (see table 13 and figure 6). The investigators explained their results as "a primary condensation of the olefinic and paraffinic part of the raw material, followed by a partial destruction of the condensation product into (a) paraffin(s) and (an) olefin(s) with different numbers of carbon atoms per molecule. The molecules formed in this way can react again with other saturated and unsaturated components of the reaction mixture."

With 1:1 ratio of isopentane to 2-methyl-2-butene, the formation of lower alkanes was competitive with a polymerization of the alkene into higher alkenes (table 14). The total yield of hydrocarbons was low. Decrease in the proportion of isopentane in the reaction mixture was considered, therefore, to be very

TABLE 12
Summary of runs carried out in lead-lined autoclave with equimolecular ratios of isoalkane to alkene
 Temperature, 20 C.; time of addition, 90 min.; time of agitation, 30 min.
 (Birch, Dunstan, Fidler, Pim, and Tait)

RUN NO.	REACTANTS		CONCENTRATION OF H ₂ SO ₄	YIELD PER CENT OF MATERIAL >27°C. ON ALKENE TAKEN	PRODUCT 27-185°C.					RESIDUE		
	Isoalkane	Alkene			On alkene taken	Specific gravity at 15.6°C.	Bromine number (Francis)	Octane rating		On alkene taken	Specific gravity at 15.6°C.	Bromine number (Francis)
								Un-leaded	+1.5 cc. T.E.L. per (Imperial) gallon			
42A	Isobutane	Diisobutene	per cent 96.9	165	per cent 136	0.705	<1	90.5	98	per cent 22	0.783	14
30 } 49 }	Isobutane	Triisobutene	96.9	163	138	0.704	<1	88.7	98.9	20.5	0.782	3.5
63	Isobutane	B.I.B. (105.5-112°C.)	97	158	135	0.707	<1	87.0	97.9	20	0.784	15
71	Isobutane	B.I.B. (95-120°C.)	97	164	137	0.703	<1	89.4	99.5	22.9	0.7815	14
26	Isobutane	Polymer gasoline (80-120°C.)	97					84.6	95.5 (+2 cc.)			
40	Isobutane	Propene*	100.6	150	122	0.698	<1	82.5	93.3	23	0.774	2.5
62	Isobutane	2-Butene	97	164	148	0.706	<1	90.2	100.3	14.8	0.785	10
97	Isobutane	1-Butene	97	159	139	0.708	2	89.1	98.1†	20.5		
73	Isobutane	Trimethylethene	97	152	121.5	0.706	1	86.1	98.5	26.5	0.786	19
77	Isopentane	Diisobutene†	97	159	120	0.712	<1	79.7	90.4	38.4	0.787	23
56	Isohexane (2-methylpentane)	Diisobutene	97	217 (after removal of unchanged isoalkane)	159.5	0.6955	<1	77.6		54.3	0.785	20

* Temperature, 30°C.

† The product was cut at 40° instead of 27° in order to remove n-pentane, originally present as impurity in isopentane.

‡ 1.0 cc. T.E.L. per Imperial gallon.

unfavorable for alkylation to isodecanes. The investigators pointed out that Birch, Dunstan, Fidler, Pim, and Tait (9) obtained much better results with a 1:1 ratio of isoalkane to alkene than those just described. This was ascribed to the manner in which the latter carried out their experiments, by dropping the alkene into a mixture of sulfuric acid and a great excess of isoalkane.

In a further study of the sulfuric acid-catalyzed alkylation, it was reported that isobutane in excess and propene gave a product consisting mainly of 2,3- and 2,4-dimethylpentanes and an appreciable quantity of 2,2,4-trimethyl-

TABLE 13

Experiment with 3 volumes of isopentane and 1 volume of "trimethylethene"
(Waterman, Leendertse, and Hesselink)

BOILING POINT °C.	YIELD (PER CENT BY WEIGHT TO THE OLEFINIC RAW MATERIAL)	n_D^{20}	d_4^{20}	$\frac{n_D^2 - 1}{n_D^2 + 2} \frac{1}{d}$	MOLECULAR WEIGHT (C ₅ H ₁₂)	BROMINE VALUE		ANILINE POINT °C.
						(a)	(b)	
16.8- 23.8	38	1.3510			73	0	0	
23.8- 26.8	30	1.3514			72	0	0	
26.8- 33.0	87	1.3540			72	0	0	
33.0- 55	9)							
55 - 63	17	1.3760	0.6567	0.3494	87	0	0	
63 -115	12)							
115 -148	73	1.4058	0.7190	0.3415	139	0	0	81.0
50 - 66 (16 mm.)	28	1.4150	0.7377	0.3394	156	0	0	82.5
66 - 90 (14 mm.)	20	1.4199	0.7457	0.3393	168	1	1	84.9
90 -120 (14 mm.)	18	1.4291	0.7663	0.3365	199	1	8	90.9
120 -140 (14 mm.)	9	1.4419	0.7891	0.3353	243	1	33	95.2*
>140 (14 mm.)	10	1.4578	0.8170	0.3339	333	1	57	102.4†

(a) McIlhiney method: total quantity of bromine consumed by the hydrocarbon mixture minus twice the quantity of bromine found in the reaction mixture in the form of acid after the reaction.

(b) Total quantity of bromine consumed by the hydrocarbon mixture without subtracting the acid titration.

* Read from graph for saturated compounds (for $r_D^{20} = 0.3353$ and molecular weight = 243): 97°C.

† Read from graph for saturated compounds (for $r_D^{20} = 0.3339$ and molecular weight = 333): 111°C.

pentane (7). Constant presence of the last hydrocarbon in the products from interaction of isobutane with 1-butene, 2-butene, or isobutene was explained on the basis of a probable isomerization of the *n*-butenes into isobutene prior to addition of the isoalkane. A less probable "rearrangement" (isomerization) of an intermediate addition product was also mentioned. The main products from the reaction of isobutane with isobutene or its lower polymers were 2,2,4-trimethylpentane and isomeric octanes, probably 2,4- and 2,5-dimethylhexanes plus 2,3,3- and 2,3,4-trimethylpentanes. Lesser products included isopentane, 2,3-dimethylbutane, 2,3- and 2,4-dimethylpentanes, 2,2,5-trimethylhexane, and probably both 2-methylpentane and 2,2,6-trimethylheptane.

The effect of *n*-butane in appreciable amounts as a diluent in isobutane-diisobutene alkylation was to lower slightly the yield of gasoline fraction boiling to 185°C. when equimolecular ratios of isoalkane to "isobutene" were used. This phenomenon is shown in table 15.

The heat of reaction in typical alkylations of isobutane catalyzed by sulfuric acid of 97.9 per cent concentration is given in table 16. An order of accuracy estimated at ± 10 per cent has been placed upon the heat of reaction figures.

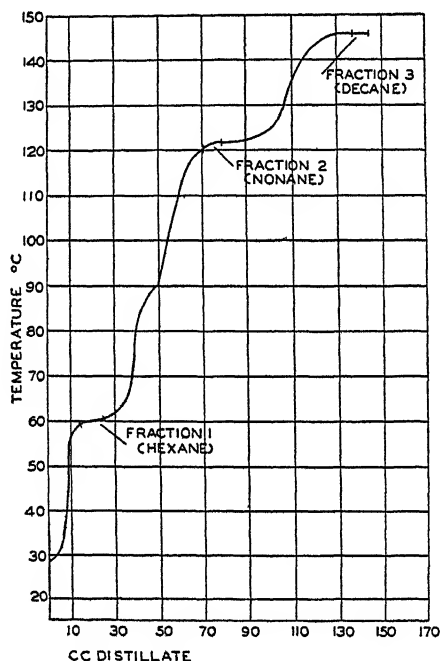


Fig. 6. Distillation of 200 cc. of a mixture of the fractions with boiling point 33°C. (at 760 mm.) to 66°C. (at 16 mm.) from experiment 1:

FRACTION	n_D^{20}	d_4^{20}	MOLECULAR WEIGHT (C_6H_{14})
1	1.3745	0.6575	87
2	1.4009	0.7079	125
3	1.4115	0.7305	138

From Waterman, Leendertse, and Hesselink.

Acid requirements to produce a definite quantity of saturated gasoline with 90+ octane rating were also considered. The conversion was considered to be no longer economical when repeated use of the acid lowers its concentration to approximately 82 per cent, corresponding to an over-all gasoline yield of 310-314 per cent by weight on the acid used. Beyond this point polymerization of the alkene replaces its function in alkylation. Addition of water to the sulfuric acid after reaction leads to the separation of highly unsaturated high-boiling

TABLE 14

Experiment with 1 volume of isopentane and 1 volume of "trimethylethene"
(Waterman, Leendertse, and Hesselink)

BOILING POINT °C.	YIELD (PER CENT BY WEIGHT TO THE OLEFINIC RAW MATERIAL)	n_D^{20}	d_4^{20}	$\frac{n^2 - 1}{n^2 + 2} \frac{1}{d}$	MOLECULAR WEIGHT (C ₄ H ₈)	BROMINE VALUE		ANILINE POINT °C.
						(a)	(b)	
19 - 27.4	22	1.3505			72	0	0	
27.4- 45	28	1.3562			79	0	0	
45 -120	12	1.3892	0.6872	0.3443	110	0	0	75.8
120 -150	19	1.4058	0.7185	0.3417	128	0	0	80.1
55 - 70 (17 mm.)	14	1.4154	0.7390	0.3391	157	1	5	81.8
70 -103 (17 mm.)	10	1.4252	0.7557	0.3385	179	4	21	84.6
103 -125 (17 mm.)	12	1.4345	0.7718	0.3377	205	6	42	87.2
125 -150 (17 mm.)	13	1.4438	0.7878	0.3370	238	8	61	90.0*
150 -175 (17 mm.)	14	1.4515	0.8044	0.3351	286	7	66	94.2†
>175 (17 mm.)	16	1.4598	0.8200	0.3339	363	3	69	102.8‡

(a) McIlhiney method: total quantity of bromine consumed by the hydrocarbon mixture minus twice the quantity of bromine found in the reaction mixture in the form of acid after the reaction.

(b) Total quantity of bromine consumed by the hydrocarbon mixture without subtracting the acid titration.

* Read from graph for saturated compounds (for $r_D^{20} = 0.3370$ and molecular weight = 38): 98°C.

† Read from graph for saturated compounds (for $r_D^{20} = 0.3351$ and molecular weight = 86): 105°C.

‡ Read from graph for saturated compounds (for $r_D^{20} = 0.3339$ and molecular weight = 63): 115°C.

TABLE 15

Effect of n-butane on the isobutane and diisobutene reaction
(Birch, Dunstan, Fidler, Pim, and Tait)

RUN NO.	ISOBUTANE CONTENT, PER CENT OF FEED ALKANES	RATIO OF ISOBUTANE TO DIISOBUTENE	PER CENT BASED ON DIISOBUTENE TAKEN	
			Gasoline (b.p. 27-185°C.)	Residue (b.p. >185°C.)
95	45	2:1	134	22.5
94	71		133	20.2
51	98		142	19.6
101	45	4.5:1	158	12.2
55	98	4:1	158	14.8
103	71	6:1	166	8.1
*	98		165	10

* These figures are interpolated from runs using isobutane:diisobutene ratios of 4:1 and 8:1.

hydrocarbons. These resemble the unsaturated oils formed along with hydro-
polymers when alkenes are hydropolymerized by sulfuric acid (31, 38, 39, 42).

The alkylation of isobutane by propene and that of isopentane by butenes were taken by Dunstan *et al.* (8) as reactions of most promise technically, giving excellent yields of high-octane-rating gasoline (table 17). Little or no addition of propene to isobutane occurred in the presence of sulfuric acid of 97 per cent initial concentration; formation of isopropylsulfuric esters and small amounts of the polymers and hydropolymers of propene resulted in its place. Dilution of the acid layer gave a small quantity of highly unsaturated oil. Acid of 100.6 or 101.7 per cent initial concentration led to the desired product, although the acid requirement was excessive. A temperature of 20°C. proved to be satisfactory. Best results were found for a 4:1 ratio of isobutane to propene; the product contained mainly 2,3- and 2,4-dimethylpentanes, besides 2,3-dimethylbutane, 2,2,4-trimethylpentane, and other isoöctanes. An interaction between isobutane and diisopropyl sulfate in the presence of concentrated sulfuric acid at

TABLE 16
Heat of reaction
(Birch, Dunstan, Fidler, Pim, and Tait)

RUN NO.	ALKENE	RATIO OF ALKENE TO ISOBUTANE	HEAT EVOLVED	
			calories per gram of alkene	B.t.u. per pound of alkene
164	Isobutene*	1:3	284	512
64, 65	Diisobutene*	1:2	122	220
66	Triisobutene*	1:12	97	175
165, 166	Unsaturated C ₄ fraction† from high-pressure crack- ing operation; total un- saturated content, 56 per cent	1:3	342	615

* The isobutane used for these reactions contained 98 per cent of isobutane.

† An isobutane concentrate containing 67 per cent of isobutane was used.

-12°C. was also observed. The product in one case had a boiling range of 88-244°C. and a bromine number (Francis) of less than 1.

Table 17 also has data on alkylations of isopentane by propene, 2-butene, and diisobutene. The reaction of isopentane with propene and 101.7 per cent sulfuric acid led to a good yield of a saturated gasoline having only a 73-octane rating. Among the identified hydrocarbons were isohexanes (2,3-dimethylbutane and probably both 2- and 3-methylpentanes) and isoöctanes (probably 2,3-, 2,4-, and 2,5-dimethylhexanes). Isopentane was found to react better with 2-butene than with diisobutene, yielding more gasoline. The gasoline had an improved volatility, higher octane rating, and a greater proportion of isononanes. It apparently contained 2-methylpentane, 2,3-dimethylbutane, much 2,2,5-trimethylhexane, and 2,2,6-trimethylheptane. Isopentane and diisobutene in 8:1 ratio gave an 82.7-octane-rating gasoline consisting mainly of C₈ to C₁₀ isoalkanes, including both 2,3-dimethylbutane and 2,2,4-trimethylpentane and probably also 2- and 3-methylpentanes, 2,4-dimethylpentane, 3-methylhexane, 2,2,5-trimethylhexane, and 2,2,6-trimethylheptane.

Another group of investigators (37) has studied (a) the alkylation of isobutane, isopentane, "isohexane" (2- and 3-methylpentanes), and 2,2-dimethylbutane as effected by sulfuric acid and a common alkene, i.e., 2-butene, and (b) similar reactions of isobutane, as a common alkane, with propene, 2-butene, isobutene, 2-pentene, 2-methyl-2-butene, octenes from 2-ethyl-1-hexanol dehydration, 2-butene dimer fraction (U.O.P. Co. polymerization process), butene dimer (hot sulfuric acid process), propene trimer (U.O.P. Co. polymerization process), diisooamylene, and butene trimer (hot sulfuric acid process). The results are summarized in tables 18-19.

Table 18 gives data on the alkylation of representative branched hydrocarbons by 2-butene. It indicates that the activity of isoalkanes decreased as the chain length increased, based upon the amount of alkylate produced per unit volume of acid. The octane rating of the alkylates likewise decreased. In the case of isopentane and 2-butene the product contained isobutane, 2-methylpentane (rather than 2,3-dimethylbutane), nonanes (probably 2,2,5-trimethylhexane and a dimethylheptane), and decanes. No alkylation of 2,2-dimethylbutane by 2-butene occurred even with a contact time of 1 hr.

Table 19 contains data on the alkylation of isobutane by representative normal or branched-chain alkenes. It indicates that a much greater percentage of lighter-boiling hydrocarbons was formed from branched-chain alkenes than from normal alkenes. With a 2-pentene feed only 28 per cent of the product boiled below the nonane range, whereas 2-methyl-2-butene gave a product containing 50 per cent of hydrocarbons boiling below the nonanes.

Alkylation of isoalkanes by alkenes in the presence of sulfuric acid for the purpose of producing 100-octane-rating aviation gasoline is practiced commercially by a number of oil companies. Their efforts have included an investigation of the underlying principles, the type of equipment used, the conditions of operation, and the results obtained with representative feed stocks (2). Studies of the following operating variables were made: ratio of isobutane to alkene, concentration of alkene in feed, ratio of acid to hydrocarbon in the reaction zone, acid strength, contact time, agitation, and temperature. Presence of a large excess of isoalkane in the reaction zone was specified. This requirement was variously met by special features in plant design. The optimum temperature for alkylation with C_4 alkenes was stated to lie between 0°C . and 10°C . A contact time of 20-40 min. was indicated for the last case, but contact times as low as 5 min. have been used. Figure 7 is a diagram of a typical sulfuric acid alkylation unit (9a).

III. THERMAL ALKYLATION

A. ALKENES

Non-catalytic addition of alkanes to ethene was established by Frey and Hepp (18). A measured proportion of propane or isobutane was circulated through copper-lined steel tubes at pressures ranging from 2500 to 4700 psi and temperatures slightly above 500°C . Ethene was injected into the hydrocarbon stream in small portions, providing a steady, low concentration of the alkylating agent. Table 20 gives data on two tests with propane and one with isobutane.

GUSTAV EGLOFF AND GEORGE HULLA

(Birch, Dunstan, Fidler, Pim, and Tait)

Run No.	40	202	73	203	77	201	231
Isoalkane.....	Isobutane	Isobutane	Isobutane	Isoptane	Isoptane	Isoptane	Isoptane
Weight taken, grams.....	1800	3240	1800	3200	2500	3520	1240
Alkene.....	Propene	Propene	Trimethyl- ethylene	Propene	Diisobutene	Diisobutene	2-Butene
Weight taken, grams.....	1300	535	2100	450	1800	650	235
Isoalkane:alkene ratio.....	1:1	4:1	1:1	4:1	2:1	8:1	4:1
Acid volume, cc.....	1800	1800	1800	1600	1600	1600	1800
Free acid concentration:							
Start, per cent.....	100.6	101.7	97	101.7	97	97	97
End, per cent.....		92.4		92.0			95.6
Crude product.*							
Per cent on alkene taken.....	150	215	152	214	159	238†	242†
Specific gravity at 15.6°C.....	0.708	0.685	0.715	0.699	0.727	0.709	0.709
Bromine number (Francis).....	1	<1	4	<1	7	2	<1
A.S.T.M. distillation, °C.:							
Initial boiling point.....	43.5	42.5	39.5	44	51.5	48	54
2 per cent.....	59.5	55.5	51	60.5	68.5	59	72
5.....	69	64	61	71.5	81	67	82
10.....	80	73	75	81.5	94.5	76.5	90
20.....	90	83.5	99.5	95.5	112.5	91.5	104
30.....	96	87	114	105	123.5	107.5	114
40.....	101.5	89	122.5	110.5	132.5	119.5	120
50.....	109	90.5	132	114	144	129	126
60.....	122	92	142	118	156	138	128.5
70.....	145.5	95	153.5	122	170	147	132
80.....	169.5	101	176	130	193.5	158	138.5
90.....	200	129	221.5	157.5	241	177	155
Final boiling point.....	248	184	282.5	201	297	236	202

ALKYLATION OF ALKANES

Gasoline:*	124	200 (to 162°C.)	121.5	205	120	216	221 (to 160°C.)
Per cent on alkene taken.....							
Specific gravity at 15.6°C.....	0.698	0.692	0.706	0.696	0.712	0.703	0.706
Bromine number (Francis).....	<1	<1	<1	<1	<1	<1	<1
Octane number (C. F. R. Motor Method):							
Unleaded.....	82.5	89.1	86.1	73.0	79.7	82.7	85.0
Plus 1 cc. tetraethyllead per Imperial gallon.....	93.3 (plus 1.5 cc.)	96.2	98.5 (plus 1.5 cc.)	83.5	90.4	91.1	95.2
Residue > 185°C.:							
Per cent on alkene taken.....	23	8.2 (>162°C.)	26.5	8.2	38.4	20	17.5 (>160°C.)
Specific gravity at 15.6°C.....	0.774	0.775	0.786	0.785	0.787	0.780	0.760
Bromine number (Francis).....	2.5	<1	19	5	23	8	1.5

* Boiling range dependent on isoalkane starting material.

† No explanation can be offered for these extremely high yields above theoretical. Careful fractionation of the product indicated the presence of not more than 2.5-3.0 per cent *n*-pentane.

Tables 21 and 22 give additional data on the composition of the liquid products produced in these runs.

Tables 20-22 show that ethene enters extensively into the alkylation of propane and isobutane. The liquid products from propane contained about 75 weight per cent of pentanes, consisting of about one-third *n*-pentane and two-thirds isopentane. About 10 per cent of heptanes was also present.

Isobutane and ethene gave a liquid product containing 56.9 weight per cent of hexanes, of which nearly 80 per cent was 2,2-dimethylbutane. The last-named hydrocarbon was pointed out as a desirable component of aviation fuel. The

TABLE 18
Alkylation of representative isoalkanes with 2-butene
(McAllister, Anderson, Ballard, and Ross)

ISOALKANE USED	ISOBUTANE	ISOPENTANE	ISOHEXANE (2- AND 3-METHYL PENTANES)	NEO- HEXANE*
Conditions:				
Mole ratio of isoalkane to alkene.	5.0	5.1	5.5	2
Strength of acid, weight per cent H ₂ SO ₄ . . .	100.0	100.0	99.8	100.0
Volume ratio of acid to hydrocarbon.....	0.7	0.7	0.7	0.6
Temperature, °C.	10	10	10	10
Contact time, minutes	20	20	20	60
Results:				
Volumes of alkylate per volume of acid. . .	20	11	5	
Weight per cent yield of alkylate, based on alkene used.....	200	264	206	None
Per cent of aviation fraction (E.P. 150° C.) in alkylate after stabilizing from original isoalkane.....	93	83	78 (E.P., 165°C.)	
Bromine number of alkylate, grams per 100 cc.....	<1	<1	<1	
Octane number (A.S.T.M.-C.F.R.) of aviation fraction of alkylate.....	94	86.5	76.5	

* Batch experiments.

reaction of isobutane with ethene also was studied from the standpoint of effect of pressure and circulation *vs.* once-through conversion. Tables 23 and 24 indicate that a temperature of 520°C. and a pressure of 2500 psi led to a fairly extensive formation of hexanes and other alkanes, although considerable thermal decomposition was evident. Tables 23 to 25 demonstrate that the hexane fraction in once-through conversion of an isobutane and ethene mixture amounted to only about one-third of that formed in recirculation with thirty-two additions of ethene (experiment No. 231-5) under otherwise similar conditions. Once-through operation also decreased the amount of 2,2-dimethylbutane present in the hexane fraction. A greater proportion of high-boiling compounds (C₈

TABLE 19
Alkylation of isobutane with representative alkenes
(McAllister, Anderson, Ballard, and Ross)

TYPE OF ALKENE USED.	NORMAL			TERTIARY		ALKENE POLYMERS					
	Pro- pane	2-Bu- tene	2-Pentene	Isobu- tene	2-Methyl- 2-butene	Octenes from 2- ethyl-1- hexanol	Propene trimer (U.O.P. Polymer)	Butene dimer (hot acid polymer)	Butene trimer (hot acid polymer)	2-Butene dimer (U.O.P.)	Diso- amylene
Conditions:											
Mole ratio of isoalkane to alkene	6.7	5.0	10.0	7.1	10.0	10.0	15.8	10.7	20.3	10.6	20
Acid strength used, weight per cent H ₂ SO ₄	98.0	100.0	98.0	98.2	98.0	98.0	100.0	98.0	100.0	97.8	100.0
Volume ratio of acid to hydrocarbon	0.7	0.7	0.7	0.7	0.7	0.7	0.7	0.7	0.7	0.7	1.0
Temperature, °C.	30	10	10	20	10	10	20	10	10	10	10
Contact time, minutes.	40	20	20	10	20	20	20	20	20	20	20
Results:											
Volumes of alkylate per volume of acid.	6	20	15 (extrap.)	12.5	14 (extrap.)	14	10.5	15 (extrap.)	11	12.5 (extrap.)	14
Weight per cent yield of total alkylate, based on alkene	213	200	185	180	192	150	193	185	166	188	203
Per cent of aviation fraction (E.P. 150°C.) in total alkylate	90	93	92	81	86	75	93	83	77	83	78
Bromine number, grams per 100 cc. of aviation fraction	<1	<1	<1	<1	<1	<1	<1	<1	<1	<1	<1
Octane number (A.S.T.M.-C.F.R.) of aviation fraction of alkylate.	88.5	94.0	91.0	91.5	93.0		78.5	93.0	93.0	88.0	91.0

TABLE 20
Products formed by interaction of alkanes and ethene under alkylation conditions
(Frey and Hepp)

Experiment No.....	231-2		231-3		231-5	
Gases reacted, weight per cent.	{ C ₂ H ₄ , 4.7 C ₃ H ₈ , 95.3		{ C ₂ H ₄ , 8.9 C ₃ H ₈ , 91.1		{ C ₂ H ₄ , 12.1 Iso-C ₄ H ₁₀ , 87.9	
Pressure, psi.....	4500		4500		4500	
Temperature, °C.....	508		510		504	
Average reaction time of alkane reactant, minutes....	3.8		4.1		4.0	
Number of alkene additions...	40		20		32	
Gasoline yield based on products recovered, weight per cent.....	7.2		11.2		16.3	
	weight per cent	mole per cent	weight per cent	mole per cent	weight per cent	mole per cent
<i>Products recovered:</i>						
H ₂			0.004	0.09	0.013	0.39
CH ₄	*		0.72	2.09	0.94	3.30
C ₂ H ₄	1.77	2.87	1.23	2.07	1.68	3.54
C ₂ H ₆	0.34	0.64	0.53	0.81	0.81	1.59
C ₃ H ₈	0.16	0.21	0.16	0.17	0.56	0.78
C ₃ H ₈	89.59	91.29	84.40	86.60	2.09	2.77
Iso-C ₄ H ₈	0.39	0.32	0.21	0.18	0.30	0.31
1-C ₄ H ₈			0.80	0.67	0.34	0.35
2-C ₄ H ₈					0.11	0.12
Iso-C ₄ H ₁₀	0.0†		0.0†		76.34	75.60
n-C ₄ H ₁₀	0.55	0.42	0.70	0.54	0.54	0.54
C ₅ H ₁₀	0.18	0.11	0.29	0.19	0.81	0.67
Iso-C ₅ H ₁₂	3.76	2.33	6.20	4.00	0.53	0.44
n-C ₅ H ₁₂	1.67	1.04	1.87	1.18	0.80	0.65
C ₆ H ₁₂	0.12	0.07	0.22	0.12	0.49	0.34
2,2-Dimethylbutane.....	0.42	0.22	0.81	0.44	7.20	4.94
2-Methylpentane.....					1.88	1.29
n-Hexane.....					0.18	0.13
Intermediate alkenes.....					0.08	0.05
Intermediate alkanes.....					0.31	0.18
C ₇ H ₁₄			0.20	0.09	0.31	0.19
C ₇ H ₁₆			1.12	0.52	0.43	0.25
C ₈ H ₁₆			0.12	0.05	0.63	0.33
C ₈ H ₁₈			0.37	0.15	1.57	0.81
C ₉ and heavier:			0.10	0.04		
Unsaturated (alkenes)....	0.16	0.07‡			0.38	0.16
Saturated (alkanes).....	0.88	0.38			0.68	0.28
Total.....	100.00	100.00	100.00	100.00	100.00	100.00

* The methane determination was lost through an accident.

† Less than 10 per cent of the butanes.

‡ Heptanes and heavier.

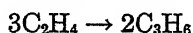
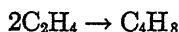
and heavier) was found; these products were more cyclic than those from the recirculation experiment. The gasoline produced in once-through operation was considered to be chiefly alkanes. It was assumed that a rapid polymerization occurs in the initial stage of the reaction by reason of the high ethene concentration and that the alkene polymers alkylate isobutane into alkanes of higher

TABLE 21
Composition of liquid products from propane and ethene
(Frey and Hepp)

Experiment No.	231-2	231-3	231-3	231-3
Gases reacted, weight per cent:				
C ₂ H ₄	4.7	8.9		
C ₃ H ₈	95.3	91.1		
Pressure, psi	4500	4500		
Temperature, °C.....	508	510		
Average reaction time of alkane reactant, minutes	3.8	4.1		
Gasoline yield, weight per cent of reactants	7.2	11.2		
	LIQUID PRODUCTS			
	Composition		Density at 25°C.	Refractive index at 20°C.
	<i>weight per cent</i>	<i>weight per cent</i>		
C ₅ H ₁₂	2.5	2.6	0.618	1.3551
Iso-C ₅ H ₁₂	52.4	55.5		
n-C ₅ H ₁₂	23.2	16.4	0.633	1.3597
C ₆ H ₁₂	1.7	2.0	0.677	1.3952
C ₆ H ₁₄	5.8	7.3		
C ₇ H ₁₄		1.8	0.706	1.3970
C ₇ H ₁₆		10.1		
C ₇ and heavier:				
Unsaturated	2.2			
Saturated	12.2			
C ₈ and above:				
Unsaturated		1.0	0.755	1.4203
Saturated		3.3		
Total	100.00	100.00		

molecular weight. However, cyclization and condensation also occurred, yielding aromatics, tar, and carbon.

In the initial stage of thermal alkylation, alkane-alkene and alkene-alkene junctures are rapid. At 500°C. and 5000 psi, 2 to 5 mole per cent of the ethene dispersed in "paraffin" is expected to follow such reactions as:



Because of the high partial pressure of the alkane used in such alkylations as $C_2H_4 + C_2H_6 \rightarrow C_4H_{10}$, these polymerizations would be surpassed by alkylation before attainment of thermodynamic equilibrium. Table 26, which is based on Kassel's calculations (32), indicates that at 500°C. a pressure of 5000 psi may be favorable for the alkylation of methane and ethane.

TABLE 22
Composition of liquid products from isobutane and ethene
(Frey and Hepp)

Test No.....	231-5		
Gases reacted, weight per cent:			
C_2H_4	12.1		
Iso- C_4H_{10}	87.9		
Pressure, psi.....	4500		
Temperature, C.....	505		
Average reaction time, minutes.....	4.0		
Gasoline yield, weight per cent of reactants.....	16.3		
Alkene in gasoline, per cent.....	16.1		
	GASOLINE		
	Composition	Density at 25°C.	Refractive index ^a at 20°C.
	<i>weight per cent</i>		
Pentenenes	4.97		
Isopentane.....	3.25		
n-Pentane.....	4.91		
Hexenes	3.01		
2,2-Dimethylbutane ...	44.26	0.645	1.3695
2-Methylpentane.....	11.55	0.655	1.3750
n-Hexane	1.11		
Heptenes.. ..	2.39		
Heptanes.....	4.54		
Octenes.....	3.87	0.714	1.3987
Octanes.....	9.64		
Residue:			
Unsaturated.....	2.33		
Unsaturated.....	4.17		
Total.....	100.00		

Additional data on the thermal alkylation of alkanes have been published (41). Table 27 gives the reaction conditions and analyses of total gaseous and liquid products for interactions of (1) ethene with propane or isobutane, (2) propene with propane, and (3) isobutene with isobutane. Analyses of the corresponding liquid products alone are given in table 28.

In table 27, experiments 2 and 5 represent a production of low-volatility alkylate from ethene with high conversion per pass; these tests meet the requirement of a greater output of gasoline from a given plant than is possible in the

synthesis of individual hydrocarbons such as 2,2-dimethylbutane. Table 28 indicates that ethene and propane yielded mainly isopentane and some *n*-pentane. Ethene with isobutane gave 2,2-dimethylbutane. Propane and propene

TABLE 23

Effect of pressure and single-point alkene addition on isobutane-ethene conversion
(Frey and Hepp)

Experiment No.....	266-11		266-12		231-5
Gases reacted, weight per cent:					
Ethene.....	16.5		9.8		12.1
Isobutane	83.5		90.2		87.9
Pressure, psi.....	4700		2500		4500
Temperature, °C.....	505		520		505
Average reaction time, minutes	5.1		4.3		4.0
Experimental method.....	Single-pass		Recirculation		Recirculation
Gasoline yield, weight per cent of effluents.	20.4		12.8		16.1
<i>Products*:</i>	<i>mole per cent</i>	<i>weight per cent</i>	<i>mole per cent</i>	<i>weight per cent</i>	<i>weight per cent</i>
Hydrogen.....	3.48	2.34	1.28	0.05	0.013
Methane.....			10.35	3.09	0.94
Ethene....	0.28	0.13	1.97	1.03	1.68
Ethane.....	5.14	2.68	4.64	2.62	0.81
Propene.....	1.05	0.76	2.21	1.72	0.56
Propane.....	3.83	2.98	3.71	3.12	2.09
Butene.....	2.35	2.26	2.22	2.32	0.75
Isobutane.....	66.14	67.97	65.35	72.53	76.34
<i>n</i> -Butane	0.44	0.45	0.64	0.71	0.54
Pentenenes	0.73	0.89	0.56	0.73	0.81
Isopentane.....	0.53	0.68	0.48	0.66	0.53
<i>n</i> -Pentane.....	3.32	4.18	1.17	1.54	0.80
Hexenes.....	0.37	0.55	0.36	0.58	0.49
2,2-Dimethylbutane.....	0.72	1.08	1.98	3.22	7.20
Other hexanes.....	1.65	2.50	1.15	1.88	2.06
Heptenes.....	0.13	0.22	0.18	0.32	0.39
Heptanes.....	0.65	1.14	0.53	1.01	0.74
Octenes.....	0.35	0.69	0.21	0.50	0.63
Octanes.....	1.76	3.52	0.48	1.03	1.57
Nonanes +	1.79	4.19	0.29	0.71	1.06
Residue.....	0.29	0.79†	0.24	0.63	
Total	100.00	100.00	100.00	100.00	100.00

* The carbon-hydrogen ratio products in tests 266-11 and 266-12 is low. This factor is sensitive to small analytical errors and will not affect largely the conclusions drawn from the analyses shown.

† 0.15 weight per cent of carbon was found in test 266-11.

yielded 2-methylpentane and 2,3-dimethylbutane. *n*-Butane and ethene were stated to produce 3-methylpentane.

The foregoing investigators pointed out that thermal alkylation bears a gen-

eral resemblance to sulfuric acid alkylation, except that the catalytic action brings about reaction at ordinary temperatures and low pressures. A difference in charging stock characteristics was considered also (41):

"The two types of alkylation are markedly different in the way different individual paraffins and olefins respond. As to the olefins, acid alkylation reacts isobutylene, 1-butenes, and propylene readily, and ethylene with difficulty. Thermal alkylation on the contrary reacts isobutylene with difficulty, *n*-butenes and propylene more readily, and

TABLE 24
Composition of liquid products from isobutane and ethene at 2500 psi
(Frey and Hepp)

Experiment No.....	266-12		
Gases reacted, weight per cent:			
Ethene.....	9.8		
Isobutane.....	90.2		
Pressure, psi.....	2500		
Temperature, °C.....	520		
Average reaction time, minutes.....	4.3		
Yield of liquid products (based on products recovered), weight per cent.....	12.8		

	LIQUID PRODUCTS		
	Composition	Yield	Density at 25°C.
	<i>mole per cent</i>	<i>weight per cent</i>	
Pentenenes.....	7.3	5.7	
Isopentane.....	6.3	5.2	
<i>n</i> -Pentane.....	15.3	12.0	
Hexenes.....	4.7	4.5	
2,2-Dimethylbutane.....	25.9	25.2	0.652
Other hexanes.....	15.2	14.7	0.682
Heptenes.....	2.4	2.5	0.714
Heptanes.....	6.9	7.9	
Octenes.....	2.8	3.9	0.741
Octanes.....	6.3	8.0	
Nonenes and heavier:			
Unsaturated.....	2.0	2.9	0.798
Saturated.....	3.3	5.0	
Residue.....	1.6	2.5	
Total.....	100.0	100.0	

ethylene most readily of all. As to the paraffins, acid alkylation is selective and limited to isoparaffins, the simplest of which are isobutane and isopentane, while thermal alkylation reacts these isoparaffins and normal paraffins with equal ease, *n*-pentane and *n*-butane reacting readily as well as propane. Ethane and methane, however, react with difficulty. In its application to gas polymerization, thermal alkylation furnishes a route for converting ethane, propane, and butane into motor fuel; ethane can be cracked or dehydrogenated to

ethylene which can be used to alkylate propane and butane in a second step. Propane or butane can also serve as an olefin source through cracking or catalytic dehydrogenation

TABLE 25
Composition of liquid products from isobutane and ethene
(Frey and Hepp)

Run No.	266-11	231-5
Charge stock, weight per cent:		
Ethene	16.5	12.1
Isobutane	83.5	87.9
Pressure, psi.	4700	4500
Temperature, °C.	505	504
Average reaction time, minutes	5.1	4.0
Experimental method.	Single-pass	Recirculation
Yield of liquid products	20.4	16.3

LIQUID PRODUCTS

	Boiling range	Composition		Density at 15.6°C.*	Refractive index at 20°C.	Composition	Density at 15.6°C.	Refractive index at 20°C.
	°C.	mole per cent	weight per cent			weight per cent		
Pentenenes		5.94	4.35			4.97		
Isopentane		4.31	3.32			3.25		
n-Pentane		27.00	20.48			4.91		
Hexenes		3.01	2.69			3.01		
2,2-Dimethylbutane	47-52	5.86	5.28	0.667		44.26	0.652	1.3695
Other hexanes	52-80	13.44	12.26	0.691		12.66	0.663	1.3750
Heptenes	80-93	1.06	1.08	0.709		2.39		
Heptanes		5.27	5.53			4.54		
Octenes	93-108	1.41	1.70	0.732	1.4108			
Octanes		8.78	10.51					
Octenes	108-117	0.58	0.70	0.746	1.4080	3.87	0.722	1.3987
Octanes		2.88	3.45			9.64		
Octenes	117-128	0.81	0.97	0.786	1.4305			
Octanes		2.73	3.27					
Nonenes +	128-205	14.56	20.50	0.793	1.4416			
Nonanes +								
Residue		2.36	3.86			6.50†		
Total		100.00	100.00			100.00		

* To convert to densities at 25°C., subtract 0.008.

† Residue boiling above 130°C. (C₉ and up).

"In combination with low-pressure cracking as a source of olefins, thermal alkylation constitutes a variant on the familiar two-stage principle, characterized by high yields and much flexibility in the use of feed stock. Ethane, propane, and butane occur together in natural gas or refinery gas and where their value as gaseous fuel is not too high, they can be exhaustively recovered and converted to produce, in such a situation, the maximum volume

of motor fuel by polymerization, by virtue of the nonselectivity of thermal alkylation with respect to conversion stock, without the addition of other conversion steps to the two-step process. Since ethane and also propane are ordinarily abundant as compared with butane in such situations, propane must be utilized in the alkylation step to make the volume of propane-butane more or less commensurate with the olefin derived from cracking ethane and the remainder of the propane. Under such circumstances the process is flexible with respect to reduction in available volume of butane. On the other hand, light straight-run or natural gasoline hydrocarbons in the normally liquid range can be included to effect reduction in volatility when desired."

A description has been given of a plant operated by Phillips Petroleum Company for the commercial production of 2,2-dimethylbutane, i.e., neohexane, from isobutane and ethene at 510°C. and 4500 psi in the alkylation coil (1). The desired temperature is easily maintained because higher temperatures bring about an endothermic cracking of isobutane. Carbon and tar formation are virtually eliminated by the high pressure used, leading to alkylation of the iso-

TABLE 26
Thermodynamic equilibria
(Frey and Hepp)

REACTION	LOG ₁₀ K (KASSEL)	K_p (500°C.)*	EXAMPLES†
(C ₄ H ₁₀)/(C ₂ H ₆)(C ₂ H ₄).....	0.296 (700 K.) -0.602 (800 K.)	0.497	C ₂ H ₄ , 2.6; C ₂ H ₆ , 5.6; C ₄ H ₁₀ , 91.8
(C ₄ H ₁₀)/(CH ₄)(C ₃ H ₈).....	-1.501 (700 K.) -2.215 (800 K.)	0.0832	C ₃ H ₈ , 5.1; CH ₄ , 16.7; C ₄ H ₁₀ , 78.2

* Computed from the values of Kassel, fugacity corrections applied.

† Compositions of equilibrium mixtures in weight per cent based on K_p values shown; pressure 5000 psi, temperature 500°C., alkene reactant 5 mole per cent of the equilibrium mixture.

butane. The ethene is slowly introduced into the reaction zone through several inlets distributed along the alkylation coil. Figure 8 is a flow diagram of the neohexane process.

B. METHYL RADICALS

An interesting transition between catalyzed and purely thermal types of alkylation of alkanes is provided in the work of Smith and Taylor (51). These investigators studied the reactions of methyl radicals (produced by photodecomposition of mercury dimethyl) with ethane, *n*-butane, isobutane, and 2,2-dimethylpropane in the temperature range 100–300°C. According to the theory given, each of the enumerated hydrocarbons (RH) is methylated by a mechanism involving a simultaneous formation of methane. Union of methyl radicals to form ethane is a competitive reaction:

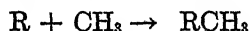
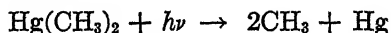


TABLE 27
*Products formed by interaction of alkanes and alkenes under alkylation conditions**
 (Oberfell and Frey)

Experiment No.....	1	2	3	4	5	6
Reactants, per cent.....	$\begin{Bmatrix} \text{C}_2\text{H}_4, 8.9 \\ \text{C}_2\text{H}_6, 91.1 \end{Bmatrix}$	$\begin{Bmatrix} \text{C}_2\text{H}_4, 25 \\ \text{C}_2\text{H}_6, 75 \end{Bmatrix}$	$\begin{Bmatrix} \text{C}_2\text{H}_6, 12.8 \\ \text{C}_2\text{H}_8, 87.2 \end{Bmatrix}$	$\begin{Bmatrix} \text{C}_2\text{H}_4, 11.8 \\ \text{Iso-C}_4\text{H}_{10}, 88.2 \end{Bmatrix}$	$\begin{Bmatrix} \text{C}_2\text{H}_4, 25.6 \\ \text{Iso-C}_4\text{H}_{10}, 74.4 \end{Bmatrix}$	$\begin{Bmatrix} \text{Iso-C}_4\text{H}_8, 8.2 \\ \text{Iso-C}_4\text{H}_{10}, 91.8 \end{Bmatrix}$
Pressure, psi.....	4500	4500	6300	4500	4500	8000
Temperature, °C.....	510	510	505	515	505	486
Total reaction time, minutes.....	4.1	5.6	7.4	2.2	4	3.2
Number of alkene additions.....	20	10	10	14	10	10
Gasoline yield based on total products.....	11.2	32.8	20.5	14.8	35.4	2.9
Analysis of products:						
H ₂	$\begin{Bmatrix} 0.004 \\ 0.716 \end{Bmatrix}$	2.29	1.59	0.55	0.84	0.12
CH ₄	1.23	0.69	0.09	1.72	2.30	0.05
C ₂ H ₄	0.53	4.10	2.64	0.71	1.44	0.04
C ₂ H ₆	0.16	0.88	2.05	0.60	0.63	0.19
C ₃ H ₈	84.40	54.17	69.29	0.76	2.04	0.05
Iso-C ₄ H ₈	$\begin{Bmatrix} 0.21 \\ 0.89 \end{Bmatrix}$	1.92	$\begin{Bmatrix} 0.89 \\ 0.85 \end{Bmatrix}$	2.04	0.98	4.10
<i>n</i> -C ₄ H ₈	0.70	3.17	2.09	78.77	56.37	92.36
Iso-C ₄ H ₁₀	0.29	1.24	1.01	0.29	0.78	0.20
<i>n</i> -C ₄ H ₁₀	6.20	9.38	$\begin{Bmatrix} 1.36 \\ 1.79 \end{Bmatrix}$	0.73	$\begin{Bmatrix} 1.11 \\ 0.97 \end{Bmatrix}$	0.11
Iso-C ₄ H ₁₂	1.82	4.22	0.82	0.77	0.95	0.05
<i>n</i> -C ₄ H ₁₂	0.22	0.80	$\begin{Bmatrix} 0.56 \\ 3.67 \\ 3.54 \end{Bmatrix}$	6.26	11.10	
2,2-Dimethylbutane.....						
2,3-Dimethylbutane.....						
2-Methylpentane.....						
<i>n</i> -Hexane.....	0.81	3.67	$\begin{Bmatrix} 1.07 \end{Bmatrix}$	$\begin{Bmatrix} 2.60 \end{Bmatrix}$	3.49	0.08

C ₇ H ₁₄	0.20	0.76	0.54	0.36	0.71	0.10
C ₇ H ₁₆	1.12	3.60	1.74	0.61	1.45	1.13
C ₈ H ₁₆	0.12	0.62	0.50	0.47	1.03	1.01
C ₈ H ₁₈	0.37	1.95	1.23	1.89	4.84	1.05
C ₉₊ to 200°C.....	0.10	{ 3.46	2.68	0.72	6.14	0.36
Above 200°C.....						
Total.....	100.00	100.00	100.00	100.00	100.00	100.00

* All percentages are by weight.

TABLE 28
Composition of liquid products* from table 27
(Oberfell and Frey)

Experiment No.	1	2	3	4	5	6
Reactants per cent.	C ₂ H ₄ , 8.9 C ₂ H ₆ , 91.1	C ₂ H ₄ , 25 C ₂ H ₆ , 75	C ₂ H ₆ , 12.8 C ₃ H ₈ , 87.2	C ₂ H ₄ , 11.8 Iso-C ₄ H ₁₀ , 88.2	C ₂ H ₄ , 25.6 Iso-C ₄ H ₁₀ , 74.4	Iso-C ₄ H ₈ , 8.2 Iso-C ₄ H ₁₀ , 91.8
Analysis of liquid products:						
C ₆ H ₁₀	2.6	3.7	5.1	1.9	2.2	6.5
Iso-C ₆ H ₁₂	55.1	28.6	6.7	5.0	3.1	3.6
n-C ₆ H ₁₂	16.2	12.9	8.8	5.2	2.7	1.6
C ₆ H ₁₂	1.9	2.4	4.4	42.1	2.7	31.3
2,2-Dimethylbutane.			2.7	17.5	9.9	2.6
2,3-Dimethylbutane.....			18.0	5.4		
2-Methylpentane.	7.1	11.2	17.7	2.4	2.0	3.2
n-Hexane.....			5.4	4.1	4.1	4.2
C ₇ H ₁₄	1.8	2.3	2.4	3.2	2.9	32.7
C ₇ H ₁₆	10.0	11.0	7.9	12.7	13.7	34.0
C ₈ H ₁₆	1.0	1.9	2.4	4.8	17.4	11.6
C ₈ H ₁₈	3.3	6.0	6.2	1.1	8.0	
C ₉ + to 200°C.	1.0	10.6	12.3			
Heavier.....		9.4				
Total.....	100.0	100.0	100.0	100.0	100.0	100.0

* All percentages are by weight.

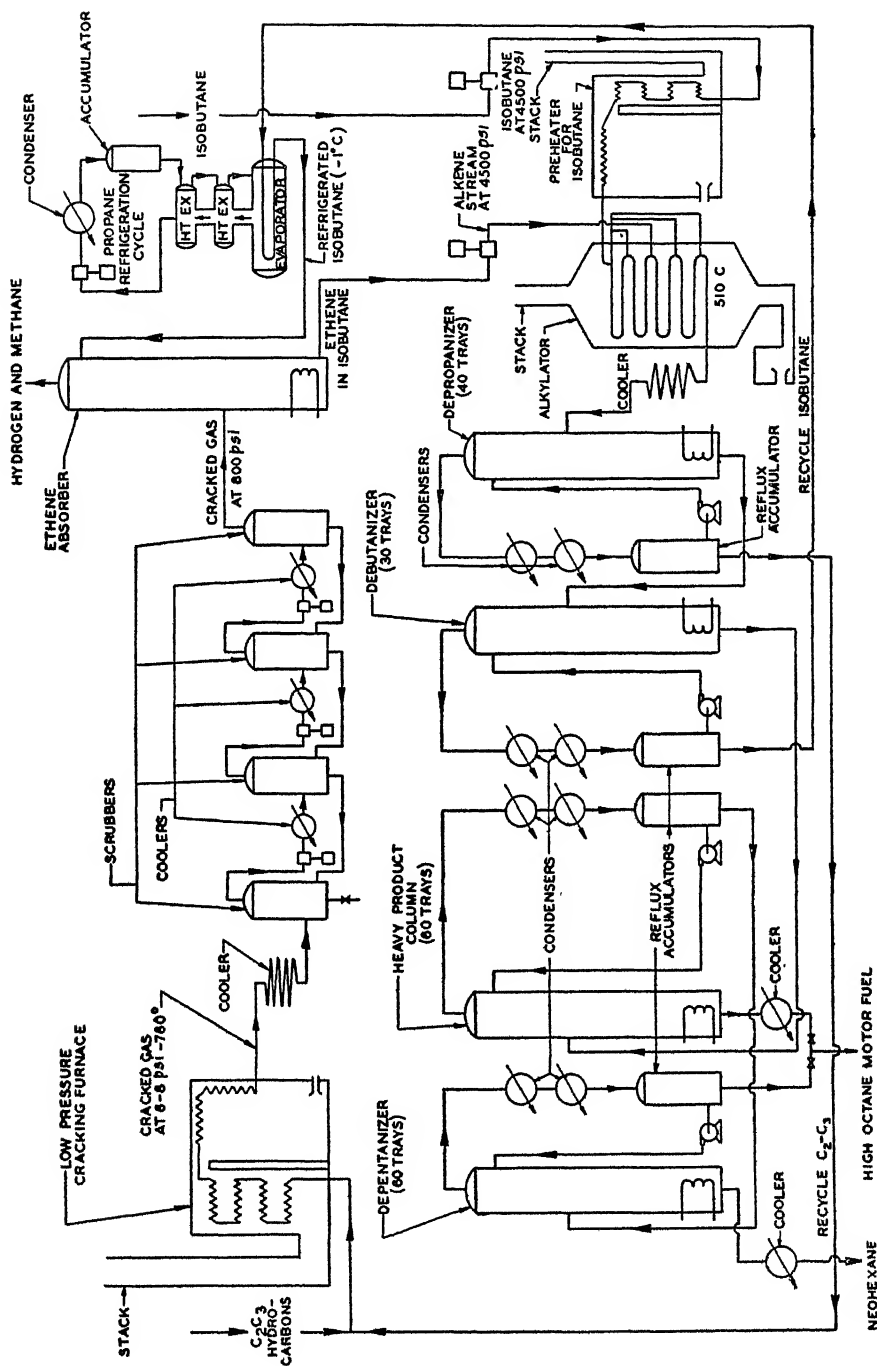


Fig. 8. Flow diagram of neohexane process (Alden)

TABLE 29
Alkylation of straight-chain alkanes

REACTANTS	PROPANE + ETHENE	PROPANE + PROPENE	<i>n</i> -BUTANE + ETHENE	<i>n</i> -BUTANE + CH ₃ Br	<i>n</i> -BUTANE + C ₂ H ₅ Br	<i>n</i> -HEXANE + ETHENE
PROCESS OR CATALYST	Thermal	Thermal	Thermal	AlBr ₃	AlBr ₃	AlCl ₃
<i>Products observed:</i>						
C ₄ H ₁₀	+	+		+	+	
<i>n</i> -Butane	+					
Isobutane	+			+	+	
C ₅ H ₁₂	+	+		+		+
<i>n</i> -Pentane	+	+				
Isopentane	+	+		+		
C ₆ H ₁₄	+	+	+		+	+
<i>n</i> -Hexane		+				
2-Methylpentane		+				
3-Methylpentane			+			
2,2-Dimethylbutane		+				
2,3-Dimethylbutane		+				
C ₇ H ₁₆	+	+				+
C ₈ H ₁₈	+	+				+
C ₉ H ₂₀	?	?				?
C ₁₀ H ₂₂	?	?				?

TABLE 30
Alkylation of isobutane by C₁-C₄ alkylating agents

ALKYLATING AGENT	METHYL BRO- MIDE	ETHENE							ETHYL BRO- MIDE	PROPENE					ISO- PROPYL FLUO- RIDE		
CATALYSTS	AlBr ₃	AlCl ₃	AlCl ₃ + HCl	NaAlCl ₄	ZnCl ₂	BF ₃ + H ₂ O	BF ₃ + HF + Ni	Solid HPO ₄	Thermal	AlBr ₃	AlCl ₃ + HCl	LiAlCl ₄	NaAlCl ₄	BF ₃ + HF	HF	H ₂ SO ₄	HF
<i>Products observed:</i>																	
C ₅ H ₁₂	+	+		+			+		+	+		+	+				+
<i>n</i> -Pentane.....		+					+		+								
Isopentane.....	+	+					+		+	+							
C ₆ H ₁₄	?	+	+	+	+	+	+	+	+	+		+	+			+	+
<i>n</i> -Hexane.....							+		+								
2-Methylpentane.....			+			+	+	+	+								
2,2-Dimethylbutane.....			+			+	+	+	+								
2,3-Dimethylbutane.....			+			+	+	+	+							+	
C ₇ H ₁₆		+		+			+		+		+	+	+	+	+	+	+
2,3-Dimethylpentane.....							+		+		+	+	+	+	+	+	
2,4-Dimethylpentane.....							+		+		+	+	+	+	+	+	
C ₈ H ₁₈		+		+	+		+		+	?		+	+	+	+	+	+
2,2,4-Trimethylpentane.....							+							+	+	+	
2,3,3-Trimethylpentane.....							+							+	+	+	
2,3,4-Trimethylpentane.....							+									?	
C ₉ H ₂₀		+		?			+		+		+	+	?				+
C ₁₀ H ₂₂		+			+		?				+	+					?
C ₁₁ H ₂₄		+															

In the cases of *n*-butane and isobutane, saturated hydrocarbons with vapor pressures in the same range as that of mercury dimethyl were observed. The reaction products from 2, 2-dimethylpropane were similarly devoid of unsaturated hydrocarbons.

TABLE 31
Alkylation of isobutane by C₄ alkylating agents

ALKYLATING AGENT	1-BUTENE	2-BUTENE		<i>n</i> -BUTENES		<i>sec</i> -BUTYL FLUORIDE	ISOBUTENE				
CATALYSTS	H ₂ SO ₄	HF	H ₂ SO ₄	AlCl ₃ + HCl	HF	HF	NaAlCl ₄	BF ₃ + HF + Ni	HF	H ₂ SO ₄	Thermal
<i>Products observed:</i>											
C ₅ H ₁₂	+	+	+			+	+	?		+	+
Isopentane.....	+		+					?		+	
C ₆ H ₁₄	+	+	+			+	+	?		+	+
2-Methylpentane	+		+							?	
2,3-Dimethylbutane.....	+		+							+	
C ₇ H ₁₆		+				+	+	+		+	+
2,3-Dimethylpentane.....										+	
2,4-Dimethylpentane.....										+	
C ₈ H ₁₈	+	+	+	+	+	+	+	+	+	+	+
2,4-Dimethylhexane.....	+		+							?	
2,5-Dimethylhexane.....	+		+	+						+	
2,2,3-Trimethylpentane...				+							
2,2,4-Trimethylpentane...	+		+	+	+				+	+	
2,3,3-Trimethylpentane...			?							?	
2,3,4-Trimethylpentane...			?	+						?	
C ₉ H ₂₀	+	+	+			+	?	?		+	?
2,2,5-Trimethylhexane....	+		+							+	
C ₁₀ H ₂₂	?	?	?			?		?		?	
2,2,6-Trimethylheptane...	?		?							?	
C ₁₁ H ₂₄								?			
C ₁₂ H ₂₆				+				+			
Higher alkanes....								+			

IV. SUMMARY OF THE PRODUCTS OF ALKYLATION

Data on the composition of products obtained in the alkylation of alkanes by catalytic or thermal methods are summarized in tables 29–33. Table 29 covers alkylations of straight-chain alkanes by such alkylating agents as ethene

and ethyl bromide. It indicates that there are definite gaps in the experimental work, including no data for methane, ethane, and *n*-pentane as reactants. Table 30 summarizes alkylations of isobutane by lower alkenes or lower alkyl halides. The data indicate that a variety of catalysts have been utilized and that not even half of the products are identified. Table 31 has data on the alkylation of isobutane by C_4 alkylating agents, including butenes and *sec*-butyl fluoride. It indicates that a number of products remain to be identified defi-

TABLE 32

Alkylation of isobutane by C_5 - C_{12} alkylating agents in presence of sulfuric acid

ALKYLATING AGENT	2-PEN- TENE	2-ME- THYL-2- BUTENE	OCTENES FROM DE- HYDRATION OF 2-ETHYL- 1-HEXANOL	"BU- TENE DI- MERS"	DIISO- BUTENE	PRO- PENE TRIMERS	DIISO- AMY- LENE	"BU- TENE TRI- MERS"	TRIISO- BUTENE
<i>Products obtained:</i>									
C_5H_{12}	+	+	+	+	+	+	+	+	+
Isopentane	+	+	+	+	+	+	+	+	+
C_6H_{14}		+	+	+	+	+	+	+	+
2-Methylpentane ..					+				?
2,3-Dimethylbutane ..		+	+	+	+	+	+	+	+
C_7H_{16}					+				+
2,3-Dimethylpentane					+				+
2,4-Dimethylpentane ..					+				+
C_8H_{18}	+	+	+	+	+	+	+	+	+
3-Methylheptane ..			+						
2,4-Dimethylhexane ..					+				?
2,5-Dimethylhexane					+				+
2,2,4-Trimethylpen- tane.	+	+	+	+	+	+	+	+	+
2,3,3-Trimethylpen- tane.	?	?	?	?		?	?	?	?
2,3,4-Trimethylpen- tane	?	?		?		?	?	?	?
C_9H_{20}	+	+			+	+			+
2,2,5-Trimethylhex- ane.					+				+
$C_{10}H_{22}$?		+		?
2,2,6-Trimethylhep- tane					?				?
$C_{12}H_{26}$?	?				+	

nately. The presence of 2,2,4-trimethylpentane among the products is known in six out of eleven instances. Table 32 covers the alkylation of isobutane by higher alkenes. In all cases the products include isopentane and 2,2,4-trimethylpentane. 2,3-Dimethylbutane is usually present also. Table 33 summarizes alkylations of isopentane and 2,2,4-trimethylpentane. The data indicate that the alkylation products have been analyzed incompletely. Hydrocarbon mix-

tures are now being analyzed by investigation of their infrared- and ultraviolet-absorption spectra, the Raman effect, and mass spectrographs. By previous methods the identification of alkanes was an exceedingly difficult task.

TABLE 33
Alkylations of isopentane and 2,2,4-trimethylpentane

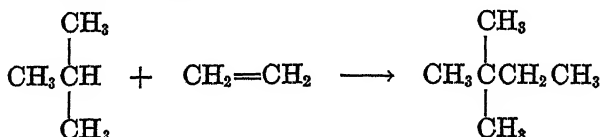
REACTANTS	ISOPENTANE								2, 2, 4-TRI-METHYL-PENTANE + ETHENE
	Ethene	Propene		2-Bu-tene	<i>n</i> -Bu-tenes	2-Methyl-2-butene	Diiso-butene	Isopropyl fluoride	
CATALYSTS	BF ₃ + HF + Ni	HF	H ₂ SO ₄	H ₂ SO ₄	H ₂ SO ₄	H ₂ SO ₄	H ₂ SO ₄	HF	BF ₃ + HF + Ni
<i>Products observed:</i>									
C ₆ H ₁₄	+		+	+	+	+	+	+	
2-Methylpentane ..			?	+	+		?		
3-Methylpentane ...			?				?		
2,3-Dimethylbutane			+	?	+		+		
C ₇ H ₁₆	+	+			+	?	?	+	
2-Methylhexane ..					+				
3-Methylhexane....							?		
2,3-Dimethylpen-tane.....		+			+				
2,4-Dimethylpen-tane		+					?		
2,2,3-Trimethylbu-tane		+							
C ₈ H ₁₈	+	+	?			?	+	+	+
2,3-Dimethylhexane		+	?						
2,4-Dimethylhexane			?						
2,5-Dimethylhexane		+	?						
2,2,4-Trimethylpen-tane							+		
C ₉ H ₂₀	+			+		+	?	+	
Dimethylheptane. ..				?					
2,2,5-Trimethylhex-ane				?			?		
C ₁₀ H ₂₂	+			+		+	?	?	+
2,2,6-Trimethylhep-tane.....				?			?		
C ₁₂ H ₂₆									+

V. MECHANISM OF ALKYLATION

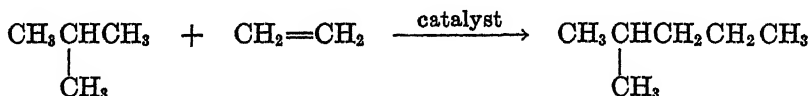
A. CATALYTIC ALKYLATION

The first published mechanism for the alkylation of alkanes is that of Ipatieff and Grosse, who postulated a direct addition of an alkene to the tertiary carbon atom of an isoalkane in the presence of a catalyst (boron trifluoride, finely divided nickel, and small quantities of either water or hydrogen fluoride) (28). The possibility of a simultaneous isomerization and the occurrence of multiple alkyla-

tion were also mentioned. They illustrated their mechanism by the direct ethylation of isobutane to form 2,2-dimethylbutane:

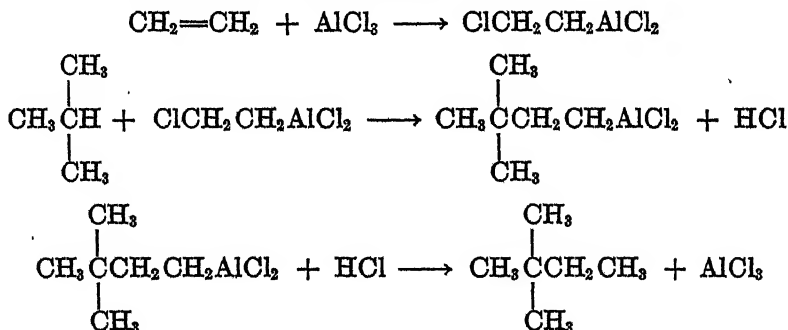


This mechanism was reconsidered in a later paper (20), wherein the hexanes produced from isobutane, ethene, and either boron trifluoride-nickel-hydrogen fluoride mixture or aluminum chloride were identified as 2-methylpentane (10-25 per cent), 2,2-dimethylbutane (<3 per cent), and 2,3-dimethylbutane (70-90 per cent). Isomerization of the 2,2-dimethylbutane was introduced therefore as an explanation of the abundant formation of 2,3-dimethylbutane and 2-methylpentane. Direct addition of ethene to a primary carbon atom of isobutane was given as an alternative explanation for the formation of 2-methylpentane.



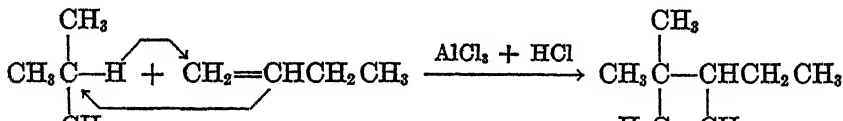
2-Methylpentane would thus be a primary product, owing to the greater availability (9:1) or more efficient catalytic activation of the primary C—H bond.

The rôle of aluminum chloride in the alkylation of isobutane by ethene was considered in still another paper (26). Isobutane was conceived as reacting probably with an aluminum chloride-ethene complex:

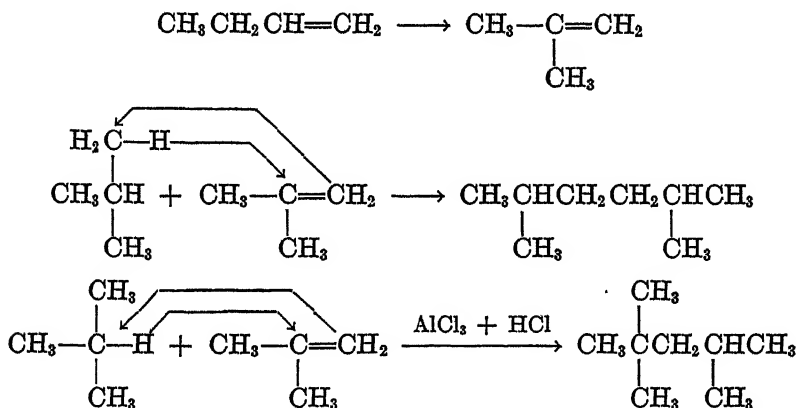


Isomerization of 2,2-dimethylbutane was considered to give 2,3-dimethylbutane.

In a similar manner, Pines, Grosse, and Ipatieff considered 2,2,3-trimethylpentane to be one of the primary products of the alkylation of isobutane by *n*-butenes (44):



Isomerization of *n*-butenes into isobutene, followed by reaction with isobutane, was taken as the probable mechanism of formation of 2,5-dimethylhexane and 2,2,4-trimethylpentane:

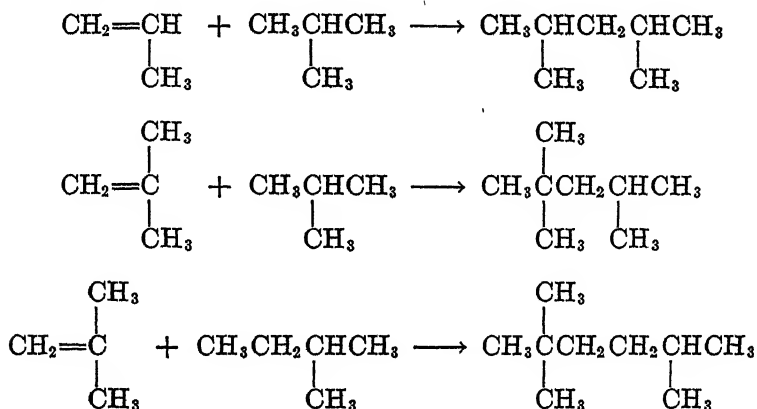


The presence of 2,3,4-trimethylpentane was explained on the basis of isomerization of the other octanes.

Heldman has pointed out the thermodynamic impossibility of an extensive isomerization of 2,2-dimethylbutane into 2,3-dimethylbutane (22). His opinion was based on the thermodynamic equilibria of Rossini, Prosen, and Pitzer (47). According to the last-named writers, the equilibrium concentrations of hexanes at 25°C. are theoretically 1.3 per cent of *n*-hexane, 7.1 per cent of 2-methylpentane, 2.5 per cent of 3-methylpentane, 84 per cent of 2,2-dimethylbutane, but only 5.4 per cent of 2,3-dimethylbutane. Even less 2,3-dimethylbutane should be present at temperatures below 25°C. A several-day isomerization of hexane over an undisclosed catalyst yielded *n*-hexane, 2-methylpentane, 3-methylpentane, 2,2-dimethylbutane, and 2,3-dimethylbutane in the proportions 4, 23, 10, 56, and 7 mole per cent for the liquid phase and 2, 18, 7, 67, and 6 mole per cent for the gas phase, respectively (32a). If the aforesaid isomerization and thermodynamic equilibria are accepted, then the amount of 2,2-dimethylbutane formed from isobutane and ethene under equilibrium conditions should be at least eight times the amount of 2,3-dimethylbutane. Evering, Fragen, and Weems indicate rapidly increasing amounts of 2,2-dimethylbutane with every drop in temperature from 204.4°C. to 21.1°C. in liquid-phase isomerization of hexanes (16a). Their experimental data for equilibrium conditions at 21.1°C. are 4 per cent of *n*-hexane, 20 per cent of 2-methylpentane, 8 per cent of 3-methylpentane, 57 per cent of 2,2-dimethylbutane, and 11 per cent of 2,3-dimethylbutane. It is probably true, however, that the time of alkylation of isobutane (10-22 hr.) was too short for the attainment of a state of equilibrium. The fact that *gem*-dimethyl structures are difficult to obtain in isomerization (15)—a result usually ascribed to steric hindrance—cannot be cited for or against the alkylation mechanism proposed by Ipatieff and Grosse (20, 26); *gem*-structured 2,2-dimethylbutane is their assumed primary product. In the *thermal* alkylation of isobutane by ethene, the principal hexane produced is 2,2-dimethylbutane.

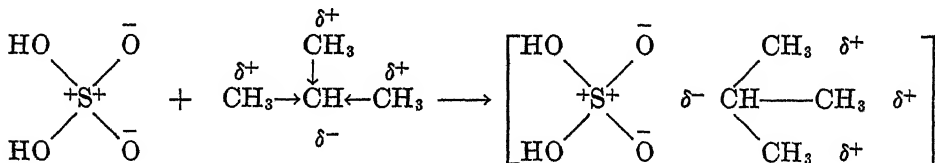
Heldman has compared the alkylation (23) of alkanes by alkyl bromides in the presence of aluminum bromide, ostensibly (22) by RAlBr_4 , with an isomerization (22, 33) of alkanes by hydrogen aluminum bromide (HAlBr_4). The common group, AlBr_4^- , is provisionally (22) considered to be the true catalyst. He predicts (22, 23) an alkylation of light alkanes along with their isomerization when the isomerization promoter is methyl bromide or possibly ethyl bromide.

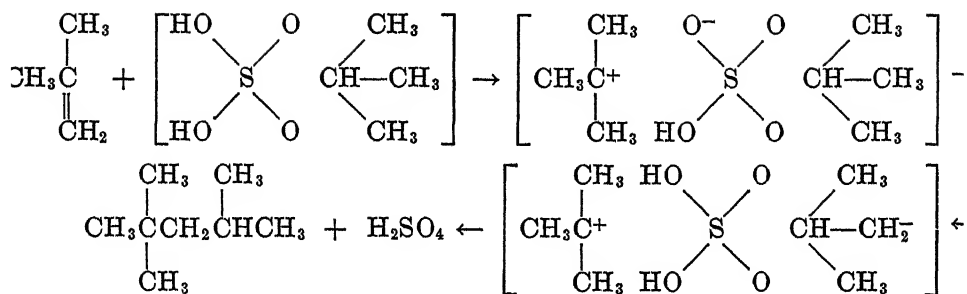
Birch, Dunstan, Fidler, Pim, and Tait have pointed out that the apparent mechanism of the alkylation of alkanes in the presence of sulfuric acid is alkylation at methyl groups (8):



An addition of alkene to the secondary and tertiary carbon atoms of the alkane, followed by isomerization, was regarded as uncertain. The presence of 2,3-dimethylbutane in the products from every alkylation examined was also disclosed; this phenomenon was not explained.

Birch and Dunstan have assumed further (6) that the alkylation proper in the sulfuric acid-catalyzed interaction of isoalkanes and alkenes involves (a) the formation of an intermediate complex between the isoalkane and the acid, (b) the transfer of a proton from the sulfuric acid in the complex to an annexing alkene molecule, which makes the latter a positive alkyl group, (c) the transfer of a proton from a *methyl* group of the alkane in the complex to the hydrosulfate group, which operation forms a negative alkyl group, and (d) the union of positive and negative alkyl groups with liberation of free sulfuric acid. These steps were illustrated for the formation of 2,2,4-trimethylpentane from isobutane and isobutene:





The electrical dipoles or formal charges associated with isobutane and its complex are those assigned by the investigators. Formation of a complex between isoalkane and sulfuric acid with the aid of dipole forces would thus be as essential as effective dispersion of the isoalkane in the acid for good yields of alkylate.

TABLE 34
Products formed in various isoalkane-alkene addition reactions
(Birch and Dunstan)

ISOALKANE	ALKENE	MAIN PRODUCTS
Isobutane	Propene	2,4- and 2,3-dimethylpentanes; 2,2,4-trimethylpentane; 2,3-dimethylbutane
Isobutane	1-Butene 2-Butene Diisobutene	2,2,4-Trimethylpentane; 2,5- and 2,4-dimethylhexanes; 2,2,5-trimethylhexane; 2,3-dimethylbutane; 2-methylpentane; (2,2,6-trimethylheptane?); isopentane
Isopentane	Propene	2,3-, 2,4-, 2,5-Dimethylhexanes; isobutane
Isopentane	2-Butene Diisobutene	2,2,5-Trimethylhexane; isohexanes (probably mainly 2- and 3-methylpentanes); (2,2,6-trimethylheptane?)
2-Methylpentane	2-Butene Diisobutene	(2,2,6-Trimethylheptane?); isobutane; isopentane

Side reactions accompanying the alkylation proper are considered to include (6): (a) depolymerization of diisobutene, triisobutene, or butene-isobutene copolymers when these are used as reactants, (b) isomerization of initial alkene, (c) hydropolymerization of initial alkene into hydropolymers and dehydropolymers, (d) dehydrogenation of initial alkane into an alkene, (e) isomerization of isoalkane produced in the alkylation proper, and (f) degradation of primary products into alkanes and alkenes. Table 34 lists the main products formed when isobutane, isopentane, or 2-methylpentane react with alkenes in the presence of sulfuric acid.

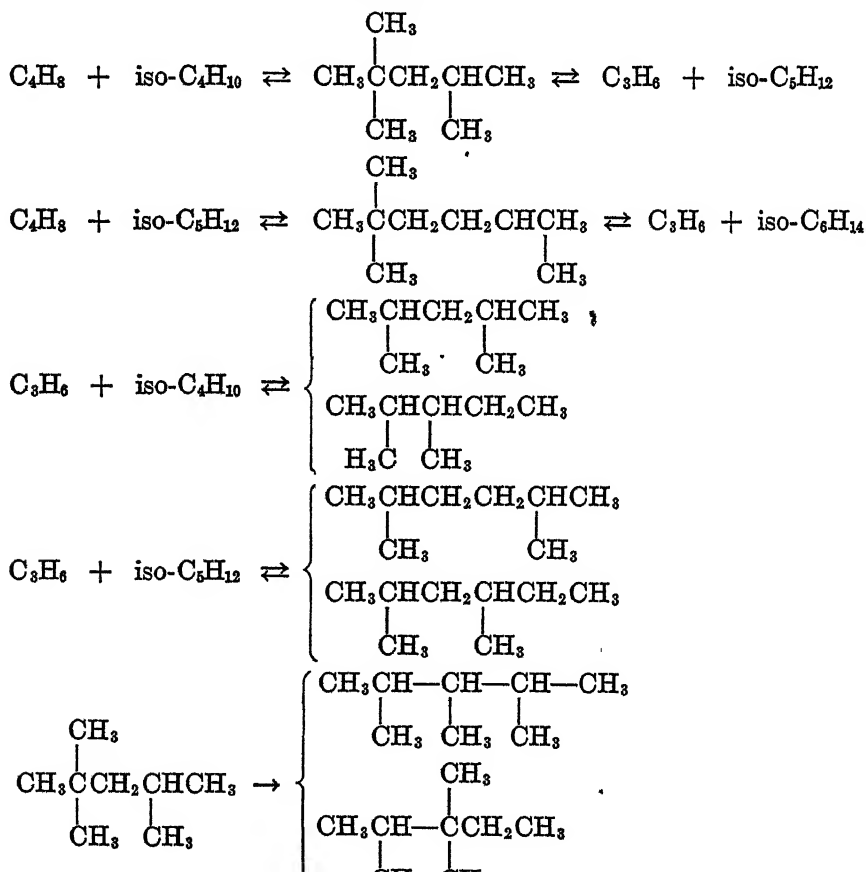
An inspection of table 34 reveals the formation of many isoalkanes with less or more carbon atoms than for an alkylation. A depolymerization of diisobutene into isobutene and isomerizations of 1-butene and 2-butene into isobutene

were assumed to be operative, accounting for closely similar, if not identical, products from isobutane in three cases, from isopentane in two cases, or from 2-methylpentane in two cases. It was suggested that the action of concentrated sulfuric acid upon 2,2,4-trimethylpentane causes a reversal of alkylation,



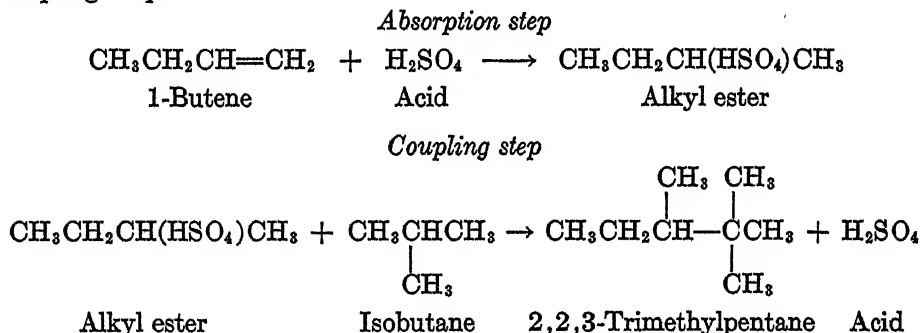
and that an observed darkening of the acid (brown coloration) is indicative of alkene formation. Other isoalkanes that were observed to break down in this way include 2,3-dimethylbutane, 2,2,3-trimethylpentane, 2,3,4-trimethylpentane, and 2,2,5-trimethylhexane. Isopentane, 2,2-dimethylbutane, and 2,2,3-trimethylbutane appeared to be unaffected by sulfuric acid; *n*-butane and diisobutene, also 2,2-dimethylbutane and 2-butene, did not enter into an alkylation and the products obtained were recognized as those from the alkenes themselves. The formation of appreciable quantities of 2,2,4-trimethylpentane in the reaction of isobutane with propene was ascribed to dehydrogenation of the isobutane into isobutene, which then reacts with the excess of isobutane present.

Formations of various by-product isoalkenes from isobutane and "butene" were explained by the following scheme:



The "butene" can contain considerable amounts of 1,3-butadiene, i.e., 22 per cent, without a noticeable adverse effect. Presumably hydrogenation of the butadiene into 1-butene and 2-butene occurs at the expense of some of the isobutane. At present only a few octanes have been isolated from isobutane-butene alkylate. From the suggested occurrence of isomerization of initial alkene and of isoalkanes from the alkylation proper, it is deducible that the iso-octanes listed as products of the alkylation can be accounted for on a qualitative basis. It would not surprise the present authors, however, if difficulties arise in the foregoing theory as quantitative data become available. For example, there are eighteen isomeric octanes whose presence or absence among alkylates must be accounted for.

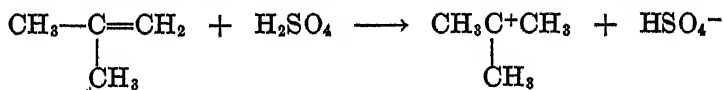
Another study of the alkylation of isoalkanes reveals that absorption of alkenes *per se* in sulfuric acid occurs about 700 times faster than that of isoalkanes without alkenes (2). The alkylation was considered to involve absorption and coupling steps:



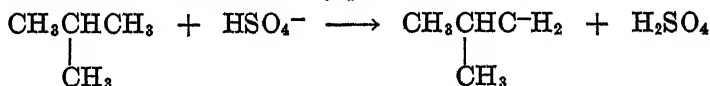
This mechanism assigns a greater initial activity of alkenes than of isoalkanes toward sulfuric acid, contrary to the requirements of the alkylation mechanism proposed by Dunstan and coworkers. The postulated 2,2,3-trimethylpentane remains to be confirmed as an actual product.

In a discussion of the paper of Birch and Dunstan (6), Waters interpreted the sulfuric acid-catalyzed alkylations of isoalkanes by alkenes as reactions of ionic type brought about by the highly polar acid (55):

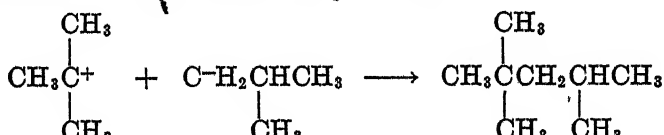
(a) Whitmore's scheme for activation of alkenes by proton addition:



(b) Ionization of the isoalkane by proton release:



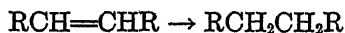
(c) Addition of the two reactive organic ions:



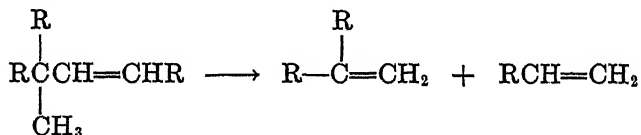
This mechanism is similar to that of Birch and Dunstan; it does not lead to different conclusions. While the formation of complexes with sulfuric acid is not postulated, transfers of a proton from the acid to the alkene and from the alkane to the proton-deficient acid (hydrosulfate ion) are assumed. Addition of carbonium ions and carbanions is the concluding step. The tertiary carbon atom of isobutane was considered to have a more electronegative environment than that possessed by an adjacent methyl group carbon atom on account of the cumulative induced polar effect of the electrical dipoles associated with the methyl groups. A group (hydrogen atom) attached to the tertiary carbon atom was taken to be more easily dissociated as an anion than as a cation (proton). Dissociation of protons from alkanes was therefore anticipated in the order $\text{CH}_3 > \text{CH}_2 > \text{CH}$. It was pointed out that methyl groups are most prevalent among isoalkanes. Because the internal polar forces (i.e., the local dipoles) in all alkanes are very small in magnitude, Waters stated, "... one would not anticipate that the relative reactivities of the various parts of any hydrocarbon molecule would be so markedly different as to necessitate the occurrence of reactions of one type only."

A markedly different type of mechanism is proposed by McAllister, Anderson, Ballard, and Ross, who postulate an addition of alkane fragments to an alkene in alkylation with sulfuric acid (37). The alkane fragments are considered to be formed by scission of C—C bonds only. A number of side reactions, such as isomerization or depolymerization of alkenes, are included, thus accounting for the observed products in a qualitative manner. Secondary alkylations traceable to a dehydrogenation of some of the starting isoalkane are also postulated; these predict a formation of 2,2,4-trimethylpentane whenever isobutane is used as part of the feed material. The following five types of reactions were considered to correspond to the structures of their observed products:

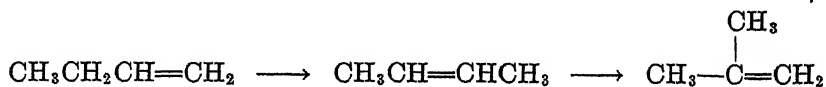
(1) Hydrogenation:



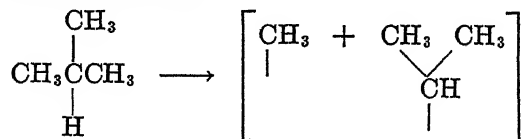
(2) Depolymerization:



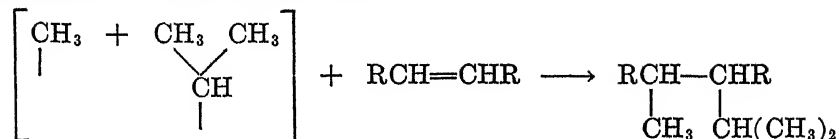
(3) Alkene isomerization:



(4) Carbon-to-carbon cleavage:



(5) Addition of fragments to alkenes:



Each of the preceding reactions involves scission of a carbon-carbon bond. Double bonds are replaced by single bonds in reactions 1, 3, and 5, whereas single bonds are broken in reactions 2 and 4. Reactions 2 and 3 require also scission of carbon-hydrogen bonds, which are thermodynamically more stable than either a single carbon-carbon bond or the π bonding of a double bond. Figures 9 and 10 illustrate the proposed mechanism of alkylation of isobutane by propene and butenes.

Table 35 gives the composition of alkylates corresponding to actual tests (table 19) and compares these with the products expected from: (a) direct alkylation, assuming that isobutane dissociates initially into methyl and isopropyl fragments, which then add to the alkene, (b) hydrogenation of the starting alkene, and (c) secondary alkylation, assuming that isobutane dissociates into methyl and isopropyl fragments and into isobutene plus hydrogen, which recombine to form higher isoalkanes (isoöctanes).

The reaction of isobutane with propene gave all hydrocarbons demanded by theory, including 2,3-dimethylbutane (figure 9). Isobutane and 2-butene gave isopentane (trace), 2,3-dimethylbutane, 2,2,4-trimethylpentane, 2,3,3- or 2,3,4-trimethylpentane, and probably some isobutane. No 2,4- or 2,5-dimethylhexane was found in the product, contrary to Birch and Dunstan (6) though in accordance with the present theory (figure 10) (compare tables 34 and 35). The propene trimer fraction did not alkylate isobutane but gave principally isoöctanes and isononanes, presumably by accepting hydrogen from half of the isobutane. In the attempted alkylation of isobutane by diisoamylene some depolymerization-hydrogenation into isopentane occurred. The main reaction was hydrogenation of diisoamylene into isodecanes at the expense of some isobutane. Isoöctanes probably were formed by the combination of excess isobutane with isobutene of dehydrogenation.

Caesar and Francis have proposed an intermolecular methyl-transfer mechanism for the low-temperature alkylation of isoalkanes by alkenes as catalyzed by metal halides or sulfuric acid (12). They state that the alkene wedges itself in between a methyl group and the rest of an isoalkane, the methyl group adding to one side of the double bond and the remainder of the isoalkane adding to the other side. Their general theory, consequently, parallels to a large extent that of McAllister *et al.* In the case of alkylation of isobutane by ethene, the general theory of Caesar and Francis accounts only for the presence of 2-methylpentane. Formation of 2,2-dimethylbutane is not provided for. An unprecedented addition of methyl and isopropyl groups across ethylidene is used to explain the observed formation of 2,3-dimethylbutane. Explanations involving similar alkylidene radicals were not used in the case of other alkylations

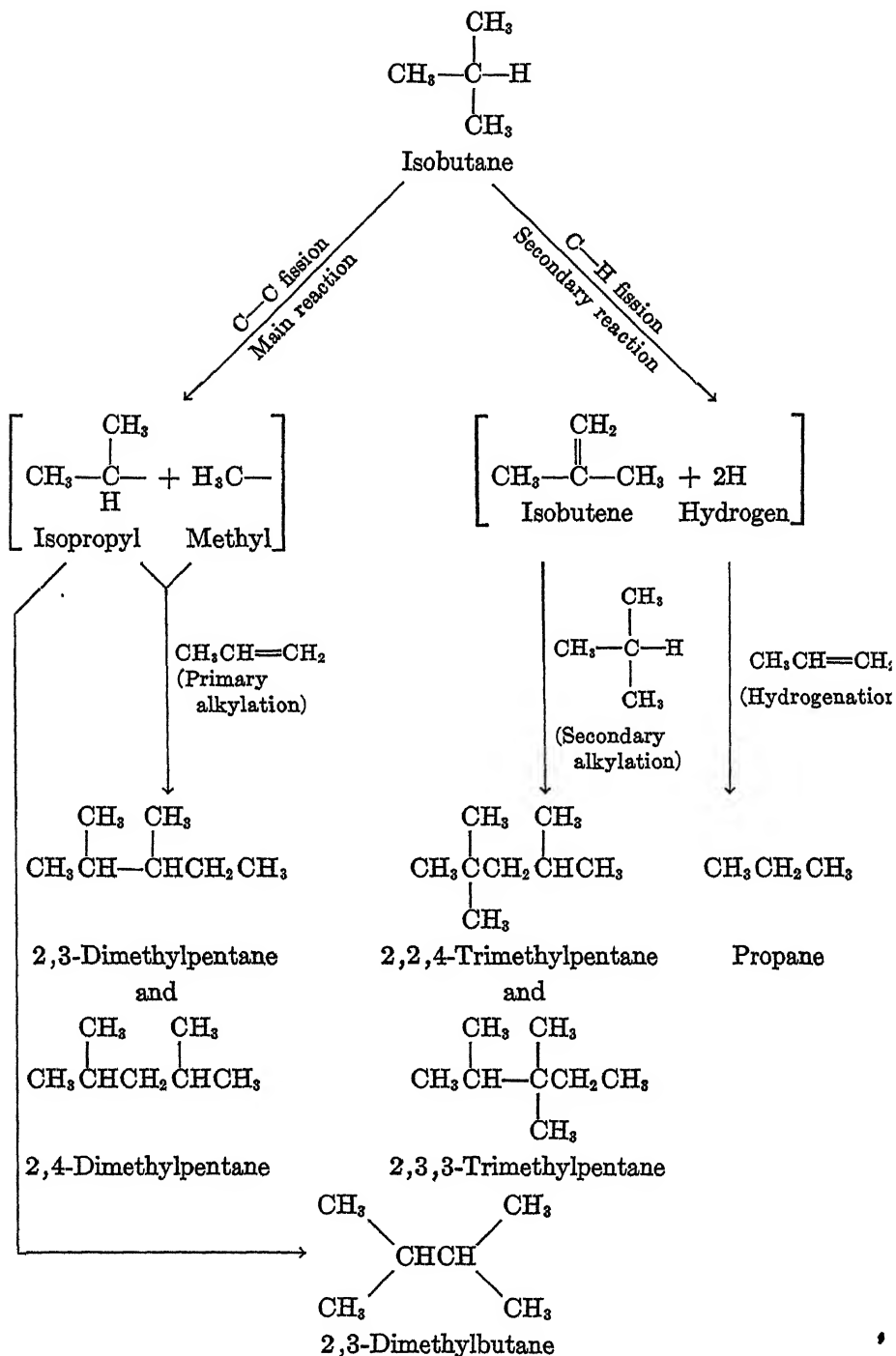


FIG. 9. Alkylation of isobutane with propene (McAllister, Anderson, Ballard, and Ros.

(table 36). Straight-chain butenes and isobutene are considered to give the same final products when they react with isobutane; this phenomenon is ascribed to a facile isomerization of the alkenes. 2,5-Dimethylhexane, formed in appreciable quantities in the sulfuric acid-catalyzed reaction (7, 9) of isobutane with isobutene, is taken as a secondary product from the similarly catalyzed reaction (8) of isopentane with isobutene to give 2,2,5-trimethylhexane.

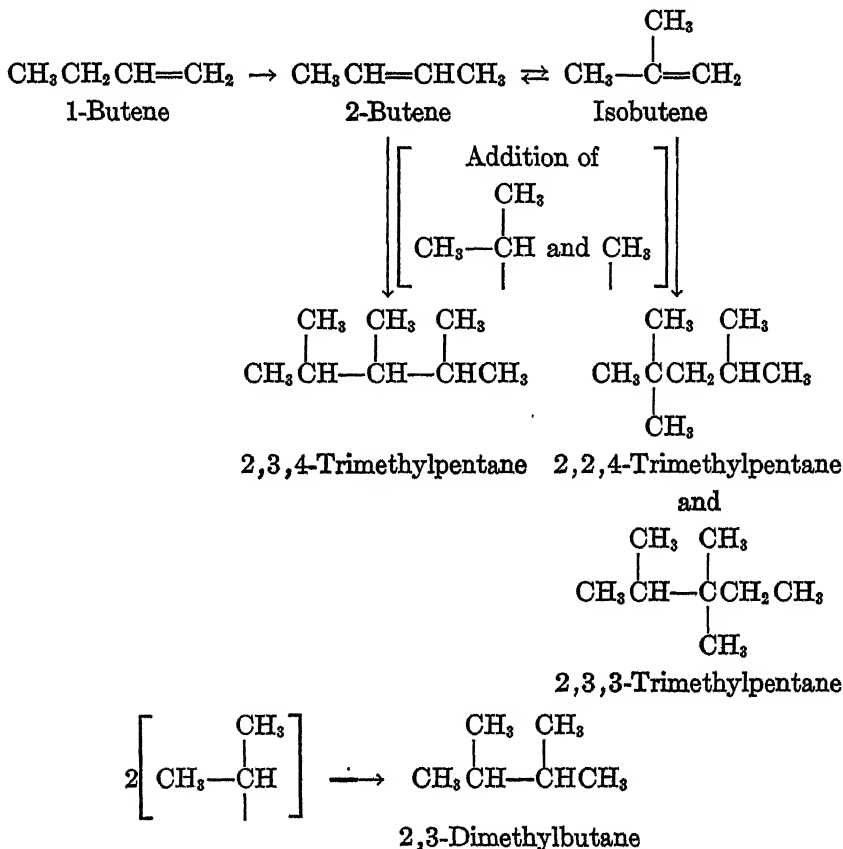


FIG. 10. Alkylation of isobutane with 1-butene, 2-butene, or isobutene (McAllister, Anderson, Ballard, and Ross).

Caesar and Francis point out that a close agreement exists between (a) the relative amounts of alkane isomers computed from thermodynamic equilibria for alkylates *without* unproposed isomers and (b) those actually formed in the low-temperature alkylation of alkanes by alkenes with metal halides or sulfuric acid (table 37).

Table 37 indicates a borderline agreement of calculated and observed data. It is difficult to believe that only two hexanes, two heptanes, or four to six octanes are present in typical alkylates. Hence, the present authors are of the opinion that the slight discrepancies between the calculated and observed data

TABLE 35
Composition of alkylation products
 (McAllister, Anderson, Ballard, and Ross)

REACTANTS	PRODUCTS OBTAINED, WEIGHT PER CENT	PRODUCTS TO BE EXPECTED FROM DIRECT ALKYLATION	PRODUCTS TO BE EXPECTED FROM HYDROGENATION	PRODUCTS TO BE EXPECTED FROM SECONDARY ALKYLATION
Isobutane and propene	2,4-Dimethylpentane 8-12 2,3-Dimethylpentane 62-66 2,2,4-Trimethylpentane 5-9 2,3,4- or 2,3,3-Trimethylpentane 6-10 Propane also formed	2,4-Dimethylpentane 2,3-Dimethylpentane	Propane	2,2,4-Trimethylpentane 2,3,3,3-Trimethylpentane
Isobutane and 2-butene	Isopentane Trace 2,3-Dimethylbutane 4-6 2,2,4-Trimethylpentane 34-38 2,3,4- or 2,3,3-Trimethylpentane 51-55 Isobutane probably also formed	2,2,4-Trimethylpentane 2,3,4-Trimethylpentane 2,3,3-Trimethylpentane	n-Butane (not found) Isobutane	2,2,4-Trimethylpentane 2,3,3-Trimethylpentane
Isobutane and 2-pentene	Isopentane 6-8 2,2,4-Trimethylpentane 6-10 2,3,4- or 2,3,3-Trimethylpentane 8-12 Isononanes 55-65	Isononanes	n-Pentane (not found) Isopentane	2,2,4-Trimethylpentane 2,3,3-Trimethylpentane
Isobutane and isobutene	Isopentane 7-9 2,3-Dimethylbutane 8-10 2,2,4-Trimethylpentane 24-28 2,3,4- or 2,3,3-Trimethylpentane 30-34 Isobutane probably also formed	2,2,4-Trimethylpentane 2,3,3-Trimethylpentane	Isobutane	2,2,4-Trimethylpentane 2,3,3-Trimethylpentane
Isobutane and 2-methyl-2-butene	Isopentane 18-20 2,3-Dimethylbutane 5-7 2,2,4-Trimethylpentane 14-16 2,3,4- or 2,3,3-Trimethylpentane 15-17 Isononanes 15-20	Isononanes	Isopentane	2,2,4-Trimethylpentane 2,3,3-Trimethylpentane

Isobutane and octenes from 2-ethyl-1-hexanol.....	Isopentane 2,3-Dimethylbutane 2,2,4-Trimethylpentane 2,3,3-Trimethylpentane 3-Methylheptane Isododecanes	3-5 3-5 12-16 (?) 35 (?)	Isododecanes	3-Methylheptane	2,2,4-Trimethylpentane 2,3,3-Trimethylpentane
Isobutane and propene trimers....	Isopentane 2,3-Dimethylbutane 2,2,4-Trimethylpentane 2,3,4- or 2,3,3-Trimethylpentane Hydrogenated trimers	2-3 1-2 15-20 18-20 45	Isotridecanes (not found)	Hydrogenated trimers	2,2,4-Trimethylpentane 2,3,3-Trimethylpentane
Isobutane and butene dimers.	Isopentane 2,3-Dimethylbutane 2,2,4-Trimethylpentane 2,3,3- or 2,3,4-Trimethylpentane Hydrogenated dimers (?) Isododecanes (?)	6-7 5-6 60-65 (?)	Isododecanes 2,2,4-Trimethylpentane* 2,3,4-Trimethylpentane 2,3,3-Trimethylpentanes	Hydrogenated dimers	2,2,4-Trimethylpentane 2,3,3-Trimethylpentane
Isobutane and butene trimers	Isopentane 2,3-Dimethylbutane 2,2,4-Trimethylpentane 2,3,4- or 2,3,3-Trimethylpentane Hydrogenated trimers	5-6 3-5 60-65 10-15	Isohexadecanes (not found) 2,2,4-Trimethylpentane 2,3,4-Trimethylpentane* 2,3,3-Trimethylpentane	Hydrogenated trimers	2,2,4-Trimethylpentane 2,3,3-Trimethylpentane
Isobutane and diisobutylene...	Isopentane 2,3-Dimethylbutane 2,2,4-Trimethylpentane 2,3,4- or 2,3,3-Trimethylpentane Isodecanes	10-12 2-3 20-25 20-25 16-20	Isotetradecanes (not found) Isononanes (not found)	Isodecanes Isopentane*	2,2,4-Trimethylpentane 2,3,3-Trimethylpentane

* From depolymerization and subsequent alkylation.

are indicative *inter alia* of the presence of small amounts of such alkanes as 3-methylpentane, 2,2-dimethylbutane, 2,3-dimethylhexane, and 2,2,3-trimethylpentane, variously reported in the present review. In the case of alkylations by 1-butene, 2-butene, or isobutene, theoretical considerations on reaction velocities indicate that the composition of alkylates should be markedly dependent on the particular butene used.

TABLE 36
Products from the alkylation of isobutane and isopentane
(Caesar and Francis)

REACTANTS	PRODUCTS*	
1. Isobutane and ethene	$\begin{array}{c} \text{C} \\ \\ \text{C}-\text{C}-\text{c}-\text{c}-\text{C} \end{array}$	$\begin{array}{c} \text{C} \quad \text{C} \\ \quad \\ \text{C}-\text{C}-\text{c}-\text{c} \end{array}$
2. Isobutane and propene	$\begin{array}{c} \text{C} \quad \text{c} \\ \quad \\ \text{C}-\text{C}-\text{c}-\text{c}-\text{C} \end{array}$	$\begin{array}{c} \text{C} \quad \text{c} \\ \quad \\ \text{C}-\text{C}-\text{c}-\text{c}-\text{C} \end{array}$
3. Isobutane and 1-butene	$\begin{array}{c} \text{C} \quad \text{C} \\ \quad \\ \text{C}-\text{C}-\text{c}-\text{c}-\text{c}-\text{c} \end{array}$	$\begin{array}{c} \text{C} \quad \text{c}-\text{C} \\ \quad \\ \text{C}-\text{C}-\text{c}-\text{c}-\text{c} \\ \text{(not positively identified)} \end{array}$
4. Isobutane and 2-butene	$\begin{array}{c} \text{C} \quad \text{c} \quad \text{c} \\ \quad \quad \\ \text{C}-\text{C}-\text{c}-\text{c}-\text{C} \end{array}$	
5. Isobutane and isobutene	$\begin{array}{c} \text{C} \quad \text{c} \\ \quad \\ \text{C}-\text{C}-\text{c}-\text{c}-\text{C} \\ \\ \text{c} \end{array}$	$\begin{array}{c} \text{C} \quad \text{c} \\ \quad \\ \text{C}-\text{C}-\text{c}-\text{c}-\text{C} \\ \\ \text{c} \end{array}$
6. Isopentane and propene	$\begin{array}{c} \text{C} \quad \text{c} \\ \quad \\ \text{C}-\text{C}-\text{C}-\text{c}-\text{c}-\text{C} \end{array}$	$\begin{array}{c} \text{C} \quad \text{c} \\ \quad \\ \text{C}-\text{C}-\text{C}-\text{c}-\text{c}-\text{C} \end{array}$
7. Isopentane and isobutene	$\begin{array}{c} \text{C} \quad \text{c} \\ \quad \\ \text{C}-\text{C}-\text{C}-\text{c}-\text{c}-\text{C} \\ \\ \text{c} \end{array}$	(other nonanes not identified)

* Carbon atoms from the isoalkane are in capital letters.

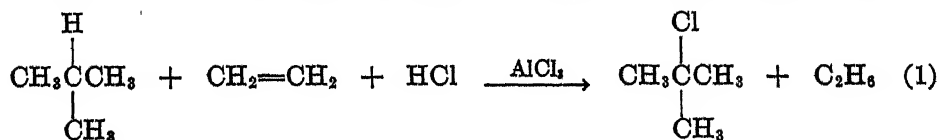
A different type of mechanism has been proposed by Schmerling (49). He suggests that alkylation of alkanes by alkenes in the presence of aluminum chloride and hydrogen chloride is a chain reaction involving transient conversion of the alkanes into alkyl chlorides. Schmerling has shown that an alkyl chloride can add to an alkene, forming a higher alkyl chloride in the absence of an alkane. Such higher alkyl chlorides are considered to exchange their chlorine for a hydro-

gen atom of the initial alkane, producing the alkylated alkanes. *Alkyl halide from the last reaction is considered to continue the process in the cyclical manner.* A preliminary formation of alkyl halide from a small portion of the alkene probably starts off the process. The reaction of isobutane with ethene to form 2,3-

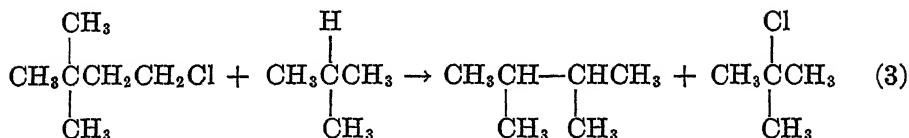
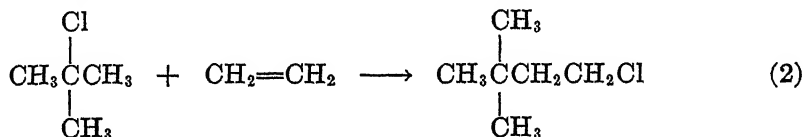
TABLE 37
Thermodynamic equilibria of isomeric alkanes at 25°C.
(Caesar and Francis)

	$\Delta F (n = 0)$	PER CENT	PER CENT IN ALKYLATE	
			Calculated	Found
<i>n</i> -Butane.....	0	32	32	20
Isobutane.....	-442	68	68	80
<i>n</i> -Pentane.....	0	4	13	10
Isopentane.....	-1097	25	87	90
Neopentane.....	-1720	71	0	0
<i>n</i> -Hexane.....	0	4	0	0
2-Methylpentane.....	-558	11	26	10-25
3-Methylpentane.....	-558	11	0	0
2,2-Dimethylbutane.....	-1341	42	0	0
2,3-Dimethylbutane.....	-1165	32	74	75-90
<i>n</i> -Heptane.....	0	0.7	0	0
One branch.....	-1035	4	0	0
2,2- and 3,3-dimethylpentanes.....	-1817	14.5	0	0
2,3-Dimethylpentane.....	-1543	9	50	50 \pm 5
2,4-Dimethylpentane.....	-1543	9	50	50 \pm 5
Triptane (2,2,3-trimethylbutane).....	-2426	40	0	0
<i>n</i> -Octane.....	0	0.3	0	0
One branch.....	-577	0.8	0	0
Gem groups.....	-1361	2.9	0	0
2,3- and 3,4-dimethylhexanes.....	-1185	2.1	0	0
3-Ethyl-2-methylpentane.....	-1185	2.1	4.7	0-10
2,4-Dimethylhexane.....	-1185	2.1	4.7	10-15
2,5-Dimethylhexane.....	-1185	2.1	4.7	
Isooctane (2,2,4-trimethylpentane).....	-2654	25	56	50-60
2,2,3-Trimethylpentane.....	-1968	8	0	0
2,3,3-Trimethylpentane.....	-1968	8	17	25-30
2,3,4-Trimethylpentane.....	-1793	6	13	
Hexamethylethane.....	-2752	30	0	0

dimethylbutane is considered to involve three main steps. First, an initiating step produces a limited, reaction-inciting quantity of *tert*-butyl chloride, probably through a chlorine-hydrogen exchange involving ethyl chloride, as follows:



Succeeding steps are taken to be those of a chain reaction utilizing and regenerating *tert*-butyl chloride:



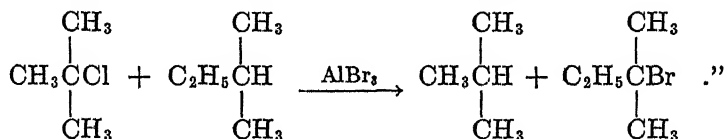
Similar reactions are assumed to occur with other isoalkanes and alkenes. Skeletal isomerization involving the neoheptyl group was compared to that characteristic of the neopentyl group, which is a major part of the neoheptyl group.

Bartlett, Condon, and Schneider (4) have demonstrated chlorine-hydrogen exchanges. They have investigated and observed the following exchange reactions of isoalkanes:

- (1) Isopentane + isopropyl chloride + $\text{AlBr}_3 \rightarrow$ *tert*-amyl bromide.
- (2) Isopentane + *tert*-butyl chloride + AlBr_3 (contact time 0.001 sec.) \rightarrow *tert*-butyl bromide (slight amount) + *tert*-amyl bromide + *tert*-amyl chloride (slight amount).
- (3) Isopentane + *tert*-butyl chloride + AlBr_3 (contact time 1 sec.) \rightarrow either 2-methylpentane or 2,3-dimethylbutane + high-boiling alkylate + *tert*-amyl bromide + 3-bromo-2-methylbutane.
- (4) Isopentane + *tert*-amyl bromide + $\text{AlBr}_3 \rightarrow$ either 2-methylpentane or 2,3-dimethylbutane + 3-bromo-2-methylbutane.
- (5) Isopentane + 4-chloro-2,2,4-trimethylpentane (diisobutylene hydrochloride) + $\text{AlCl}_3 \rightarrow$ 2,2,4-trimethylpentane + *tert*-amyl chloride + nonyl chlorides.
- (6) 3-Methylpentane + *tert*-amyl bromide + aluminum halide \rightarrow isopentane + bromohexanes.
- (7) 2,3-Dimethylbutane + *tert*-butyl chloride + $\text{AlCl}_3 \rightarrow$ 2-chloro-2,3-dimethylbutane.
- (8) 2,2,3-Trimethylbutane + *tert*-butyl chloride + $\text{AlCl}_3 \rightarrow$ 3-chloro-2,2,3-trimethylbutane.
- (9) 2,2,4-Trimethylpentane + *tert*-butyl chloride + $\text{AlCl}_3 \rightarrow$ products distilling 31–103°C. (no chlorotrimethylpentane).

In the case of the isopentane, *tert*-butyl chloride, and aluminum bromide mixture, the fastest reaction was probably a chloride-bromide exchange affecting the *tert*-butyl chloride. The slight amount of *tert*-butyl bromide so formed disappeared within 1 sec. Within the same time interval, a considerable portion of the *tert*-amyl bromide initially formed from the isopentane was isomerized to 3-bromo-2-methylbutane. Also, there developed the first indication of low- and high-boiling "alkylation" products. Thus the first distinctly recognizable

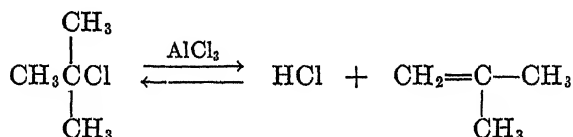
reaction between isopentane and *tert*-butyl chloride with aluminum bromide is "an extremely rapid *halogen-hydrogen exchange* in which the original paraffin is converted into halide and the original halide into paraffin:



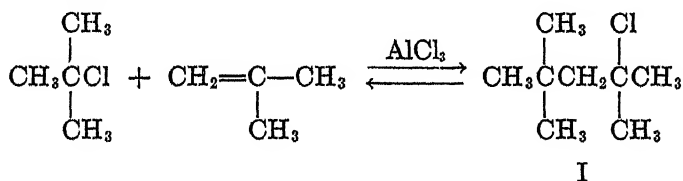
The resulting *tert*-amyl bromide was isolated in 50–70 per cent yields. No halogen–hydrogen exchange occurred when *n*-pentane was substituted for isopentane. A chlorotrimethylpentane was absent from the products of an interaction between 2,2,4-trimethylpentane, *tert*-butyl chloride, and aluminum chloride, although a related reverse reaction occurred normally (see exchange reaction 5, in which isopentane was used instead of isobutane because of more convenient boiling point).

Bartlett and coworkers (4) have discussed Schmerling's mechanism of alkane alkylation in terms of carbonium ions or "active fragments with an electron-deficient carbon atom," leaving open the question of whether the lifetime of the intermediate is such as to justify the use of the term "ion." Bartlett accounts for "normal" products of alkylation by the following sequence of reactions in accordance with Schmerling's mechanism, using the alkylation of isobutane by *tert*-butyl chloride and aluminum chloride as an example:

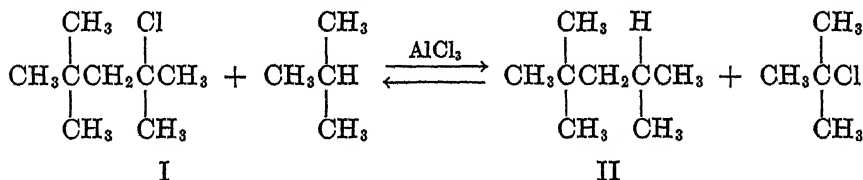
(1) Reversible elimination of hydrogen halide:



(2) Addition of halide to alkene:

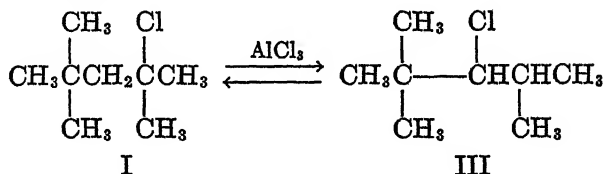


(3) Halogen–hydrogen exchange:

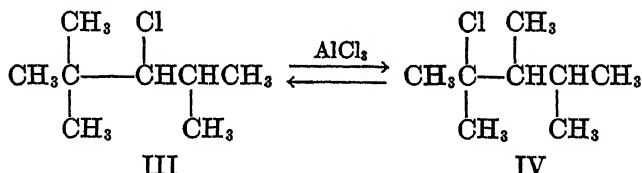


2,2,4-Trimethylpentane

(4) Partial isomerization (analogous to that observed with *tert*-amyl bromide and aluminum bromide):

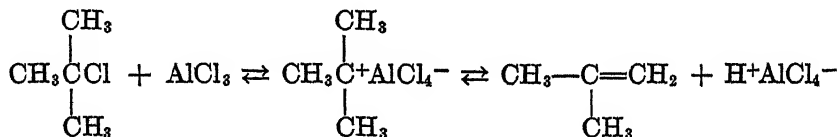


(5) Wagner-Meerwein rearrangement:

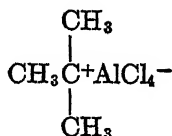


Product IV upon halogen-hydrogen exchange would give some 2,3,4-trimethylpentane, which will be designated as V. The latter was stated to be "subject to further halogen-hydrogen exchanges and isomerization from which 2,3,3- and 2,2,3-trimethylpentanes can be derived strictly in accordance with demonstrated analogy."

Detailed mechanisms in terms of "carbonium ions" were then presented for the sequence of reactions leading to "normal" products. Reaction 1 (reversible elimination of hydrogen halide) was taken as a very rapid equilibrium because of the highly ionic complexes formed between aluminum halides and organic halides:

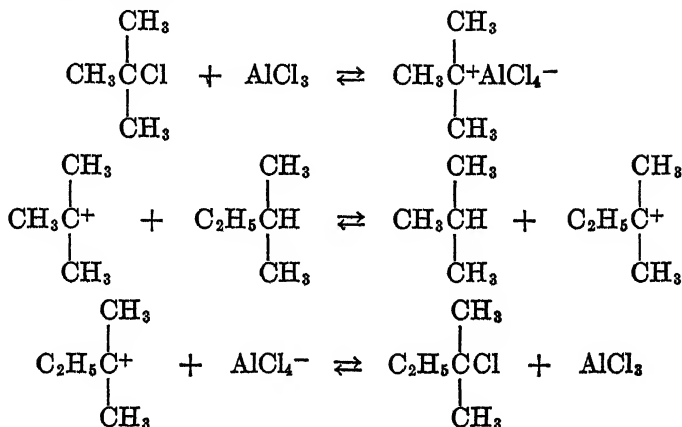


Reaction 2 (addition of halide to alkene) was postulated to proceed by way of the ionic complex,

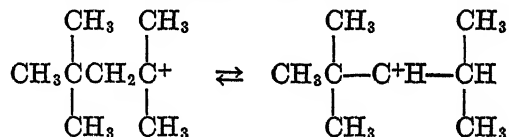


in the manner of an ionic mechanism of polymerization. Reaction 3 (halogen-hydrogen exchange), which is competitive with isomerization, rearrangement, and "depolymerization" (cracking) in the presence of aluminum halides, was considered to be a process in which an actual or virtual alkyl ion (from an ionic complex such as I + AlCl₃) collides with an alkane. The latter supplies the alkyl ion with a hydrogen particle and a pair of bonding electrons. The cor-

responding reaction between isopentane and *tert*-butyl chloride was illustrated by the following equations:

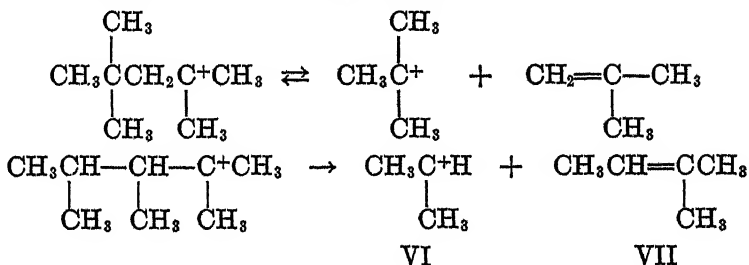


Reaction 4 (partial isomerization of alkyl halide) was formulated as an elimination and readdition of hydrogen halide or of chlorine ions, although "it is not at all certain that a proton needs to leave the carbonium ion in order to bring about the isomerization." Thus a reversible isomerization of carbonium ions



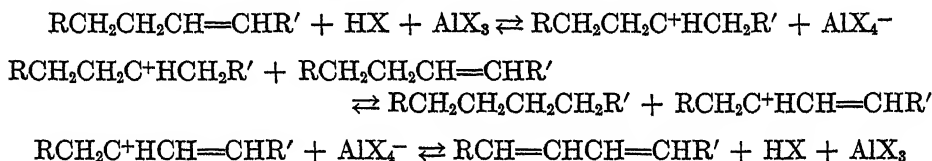
would permit a direct shift of a hydrogen with its electron pair from one carbon to the next. Reaction 5 was pointed out as a practically irreversible isomerization of a "pinacolyl" type halide (III), leading to the formation of 2,3,4-trimethylpentane (V) and isomers therefrom in competition with formation of 2,2,4-trimethylpentane (II of reaction 3).

"Abnormal" products encountered in alkylation were accounted for on several bases. One (mentioned only because it had not been definitely disproved) was the direct transfer of an alkyl group, presumably with its pair of bonding electrons, from one hydrocarbon to another. A second explanation was cleavage of a larger molecule into fragments. In particular, ionic cleavage of carbonium ions from I and IV (precursors of 2,2,4- and 2,3,4-trimethylpentanes) would give normal and abnormal fragments, respectively:



The isopropyl positive fragment (VI) would immediately attach itself to an isobutene molecule, forming 2,4-dimethyl-1-pentene, which would yield the corresponding alkane upon addition of hydrogen chloride followed by chlorine-hydrogen exchange. In a similar manner, 2-methyl-2-butene (VII) could produce polymers, copolymers, or isopentane.

The reaction of isopentane with isobutene and aluminum bromide was presented as evidence that formation of an alkene polymer is an intermediate step in the formation of "abnormal" products in low-temperature alkylation. Reactants precooled to 0°C. gave polyisobutene (contact time 0.2 sec.), but a typical alkylate appeared at room temperature with a contact time of 105 min. This gives support to the idea that the alkylate consists largely of cleavage products of polyisobutene. A similar mechanism could not be given for alkylations of isobutane by ethene or propene in which "abnormal" products are notably less abundant. Formation of highly unsaturated hydrocarbons (reaction residues) in alkylations using aluminum halide were explained by the scheme:



Resonance would stabilize the conjugated hydrocarbons produced according to the last equation.

In a longer paper following his preliminary communication (49) to the Journal of the American Chemical Society, Schmerling has recently discussed his chain mechanism for alkane alkylation in more detail. The alkylation of isoalkanes by alkenes is considered to involve the following key reactions (50) analogous to those previously outlined: (1) conversion of the isoalkane to a *tert*-alkyl ester; (2) addition of the *tert*-alkyl ester to the alkene to yield an ester of higher molecular weight; (3) reaction of the ester of higher molecular weight with the isoalkane to yield the observed alkane product and a new molecule of the *tert*-alkyl ester.

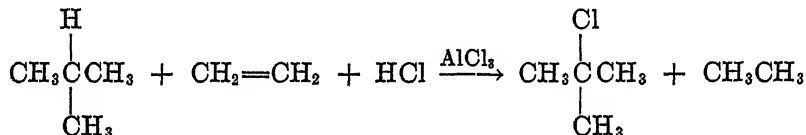
The *tert*-alkyl ester formed in the third step reacts with alkene as in the second step and the cycle is repeated. The possible separate occurrence of each step in the mechanism has been proved only in the case of the aluminum chloride reaction. Since the alkylation products obtained with other catalysts, including sulfuric acid and hydrogen fluoride, are similar to those obtained with aluminum chloride, Schmerling presumes that analogous reactions occur with these other catalysts, the reaction intermediates being the *tert*-alkyl hydrogen sulfates and *tert*-alkyl fluorides, respectively, rather than the *tert*-alkyl chlorides. He states: "... it seems desirable to emphasize the key steps of the reaction chain, to give experimental 'molecular' proof of their feasibility, and to abstain in so far as possible from expressing the mechanism in terms of ions or other inner underlying motivators. It is quite probable that catalytic alkylation is motivated by the same forces that cause other catalytic reactions."

Hydrogen-chlorine exchange reactions (reactions 1 and 3) and the addition of *tert*-butyl chloride to an alkene (reaction 2), for example, are presumed to proceed by either a molecular or an ionic mechanism, either with or without the intermediate formation of an aluminum chloride complex, such as alkyl aluminum tetrachloride, RAlCl_4 . The function of hydrogen chloride as promoter for aluminum chloride in the alkylation of isoalkanes is presumed to be the initiation and maintenance of the formation of a *tert*-alkyl halide according to reaction 1. Also, it might convert the metal halide to the more active acidic form, HAlCl_4 . It is pointed out that present-day theory favors the ionic scheme; rearrangement which occurs during the last exchange reaction (reaction 3) was taken as especially well explained by Whitmore's "common basis of intramolecular rearrangements" or the so-called "carbonium ion" theory.

An insight into the causes for the relative difficulty with which normal alkanes are alkylated was given. The first exchange reaction (reaction 1) does not take place as easily with hydrogen atoms attached to secondary carbon atoms (see page 385) as it does with those attached to tertiary carbon atoms. Under the conditions required to obtain the hydrogen-halogen exchange reaction with normal alkanes, polymerization of the alkene becomes the predominant reaction.

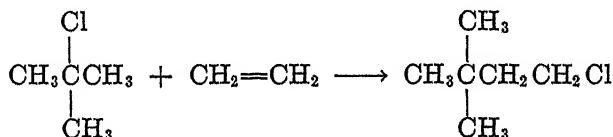
Additional evidence was presented for the three key steps:

For equation 1:



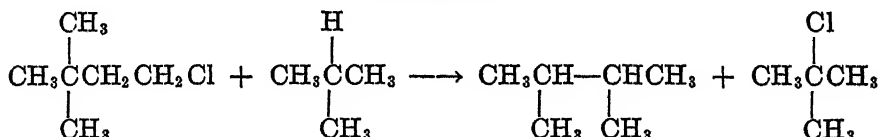
Ethene and hydrogen chloride probably form ethyl chloride. The latter is reduced to ethane by reaction with *n*-heptane in the presence of aluminum chloride (40). At the same time, the heptane is converted largely into cyclanes and into unsaturated hydrocarbons combined with the catalyst in the so-called "lower layer." Ethane and propane have been obtained as by-products of the aluminum chloride-catalyzed alkylation of isobutane with ethene and propene, respectively (53). Propane has been isolated from the products of an alkylation of isobutane by propene in the presence of sulfuric acid (37). The reaction of isobutane with isopropyl chloride gives propane, octanes, and catalyst complex (53). When a solution of aluminum bromide in isopentane is brought into contact with isopropyl chloride or *tert*-butyl chloride at room temperature, one of the principal products is *tert*-amyl bromide (4).

For equation 2:



"An investigation [48a] of the condensation of alkyl halides with olefins in the presence of metal halide catalysts has shown that the primary reaction is that of addition of the alkyl group and the halogen atom to the double bond of the olefin. For example, a 75% yield of 1-chloro-3,3-dimethylbutane is obtained by the addition of *t*-butyl chloride to ethylene in the presence of aluminum chloride at -15° to -10° [48a]. The reaction of *t*-butyl chloride with other olefins occurs in an analogous manner. The primary product of the reaction with propene, for example, is 2-chloro-4,4-dimethylpentane; under some conditions this is isomerized in part to 2- and 3-chloro-2,3-dimethylpentane."

For equation 3:



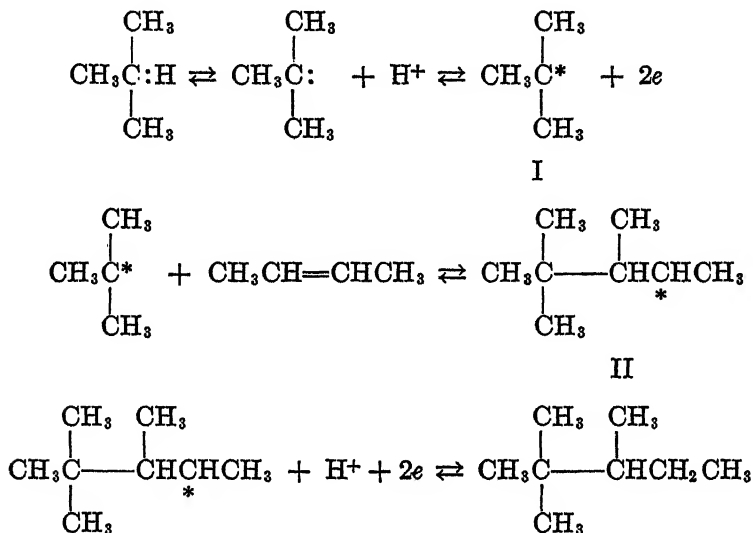
"The reaction involved in this step is essentially the same as that in Eq. 1, the chief difference being that in this case the isobutane reacts with an alkyl chloride of higher molecular weight. Excellent evidence that such chlorides are reduced to paraffins in the presence of isobutane may be found in the results obtained by the condensation of isobutane with allyl chloride in the presence of aluminum chloride [48]. At low temperatures (below 0°) the chief product is 1-chloro-3,4-dimethylpentane. At higher temperatures interaction of this chloroheptane with excess isobutane results in the formation of heptane and other paraffinic hydrocarbons, the latter being produced by secondary reactions of the intermediate *t*-butyl chloride.

"Direct proof that rearrangement of the carbon skeleton of the alkyl chloride occurs during its conversion to paraffin was obtained by showing that 2,3-dimethylbutane and *t*-butyl chloride are major products of the reaction of 1-chloro-3,3-dimethylbutane with isobutane in the presence of aluminum chloride at 22° ; relatively little 2,2-dimethylbutane is formed. The rearrangement is not unexpected since a neopentyl system is involved [56]. In some cases, as for example, in the formation of 2,3-dimethylbutane from ethylene or of 2,3-dimethylpentane from propene, the migration of the methyl group is preceded by the migration of one of the methylene hydrogens of the neopentyl group. In other cases, as for example in the formation of 2-methylpentane from ethylene or of 2,4-dimethylpentane from propene, the methyl group undergoes an α,γ -shift.

"Inferential evidence that the hydrogen-chlorine exchange step occurs during alkylation may be obtained from the fact that dichloroalkanes are produced by the low-temperature condensation of isobutane with vinyl chloride and allyl chloride in the presence of aluminum chloride [48]. These dichlorides are formed by the addition of *t*-butyl chloride to the chloro-olefins in a manner similar to the reaction of Eq. 2. In the case of vinyl chloride, the resulting 1,1-dichloro-3,3-dimethylbutane undergoes the reaction analogous to Eq. 3 in only a very small amount; in other words, the condensation is largely "frozen" at the end of the second step, and the dichlorohexane is the chief product. In the case of allyl chloride, on the other hand, the 1,2-dichloro-4,4-dimethylpentane contains a secondary chlorine atom and the major portion reacts with isobutane to yield 1-chloro-3,4-dimethylpentane (the chief product) and *t*-butyl chloride. The isolation of the dichloroalkanes may be used as an argument in favor of employing a 'molecular' rather than an ionic reaction scheme."

Ciappetta, also, has applied carbonium-ion theory to the alkylation of isoalkanes by alkenes in the presence of a catalyst such as concentrated sulfuric acid (13). The isoalkane is considered to form a carbonium ion through "ionization

and partial dehydrogenation," i.e., through loss of a proton followed by elimination of a pair of electrons. Reaction of this carbonium ion with the alkene is then assumed to give a higher carbonium ion, which would produce the corresponding alkane on hydrogenation. The proposed mechanism was illustrated by the alkylation of isobutane with 2-butene (an asterisk indicates the carbon atoms deficient in electrons):

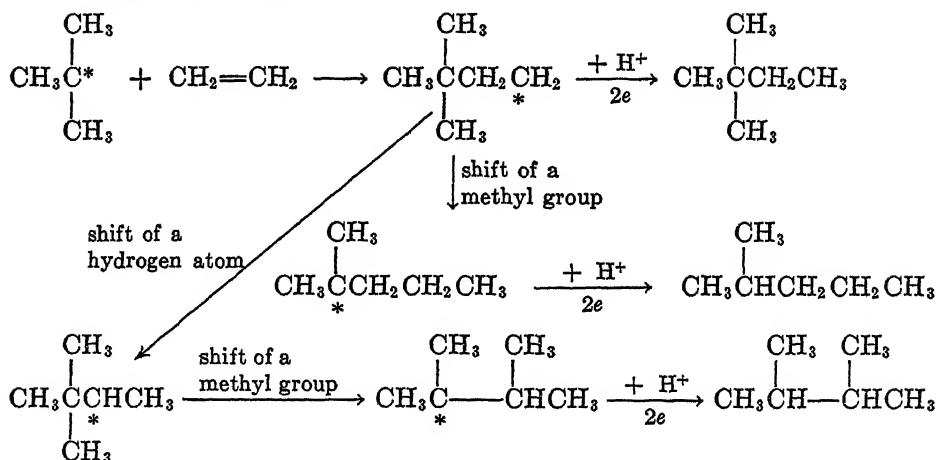


Formation of positive *tert*-butyl ions from isobutane in two steps *via* a negative *tert*-butyl ion is regarded by the present authors as improbable. In particular, the liberation of free electrons by a carbanion can scarcely be correct. The proposed mechanism thus requires revision and cannot be accepted in its present state. In this respect, the chain mechanism proposed by Schmerling has the advantage that it gives a plausible explanation for the continued formation of a *tert*-butyl ion or ester; the second step of Ciapetta's mechanism corresponds to the second or alkylation step discussed by Schmerling and accepted by Bartlett, Condon, and Schneider.

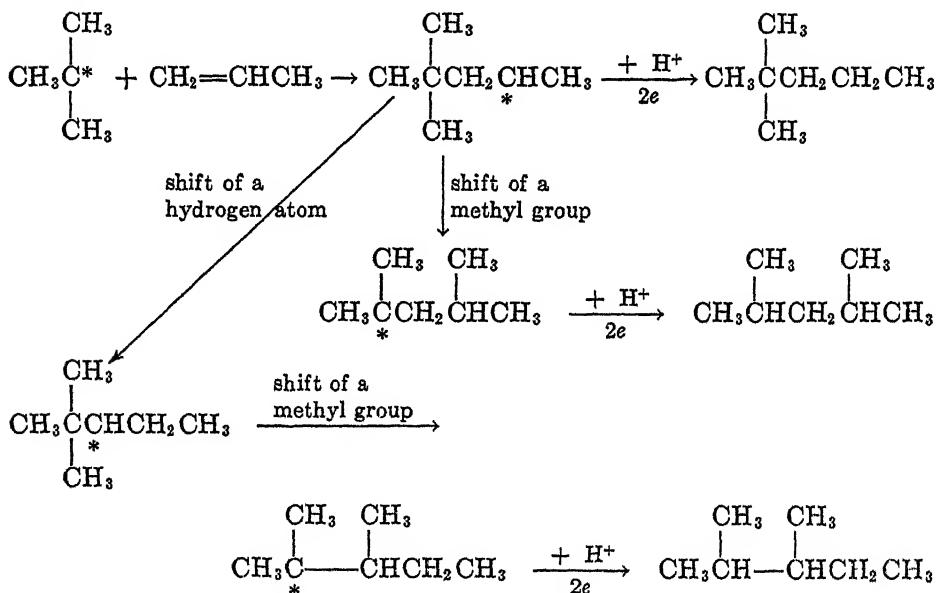
Carbonium ion II was taken as the source of primary and secondary products observed experimentally. Thus, rearrangement of II caused by the shift of a methyl group, alone or with a hydrogen atom together with their bonding electron pairs, explains many of the primary products. "Depolymerization" of II would lead to many of the observed secondary products. The assigned mechanism is in agreement with (a) the molecular hydrogenation-dehydrogenation properties of sulfuric acid and (b) the similarity of carbon skeletons of hydrocarbons produced in the reaction of *n*-butenes with isobutane (alkylation) or with isobutene (copolymerization in less concentrated acid).

The alkylations of isobutane with ethene, propene, isobutene, 1-butene, and 2-butene were depicted as follows (14):

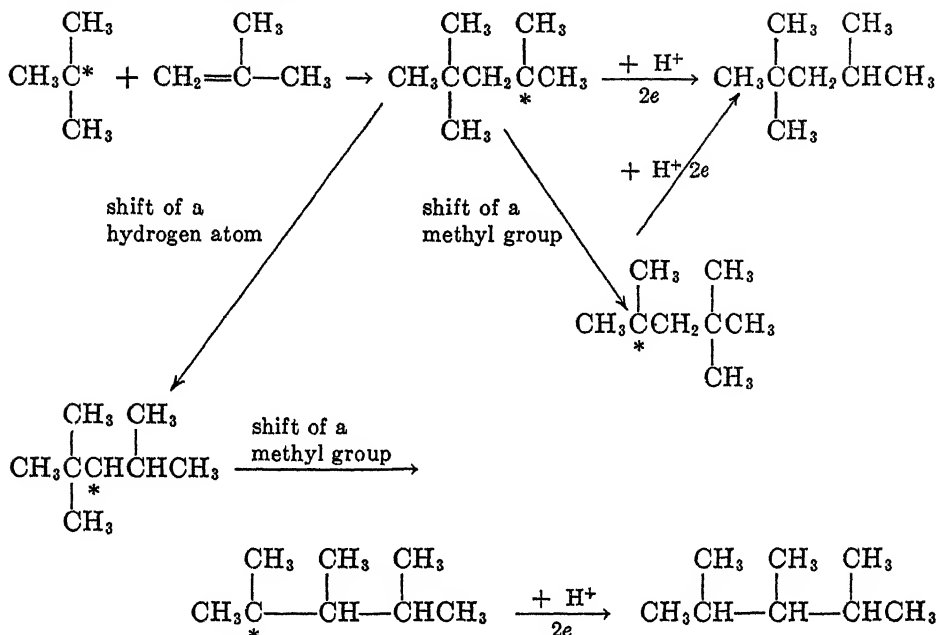
1. Reaction with ethene:



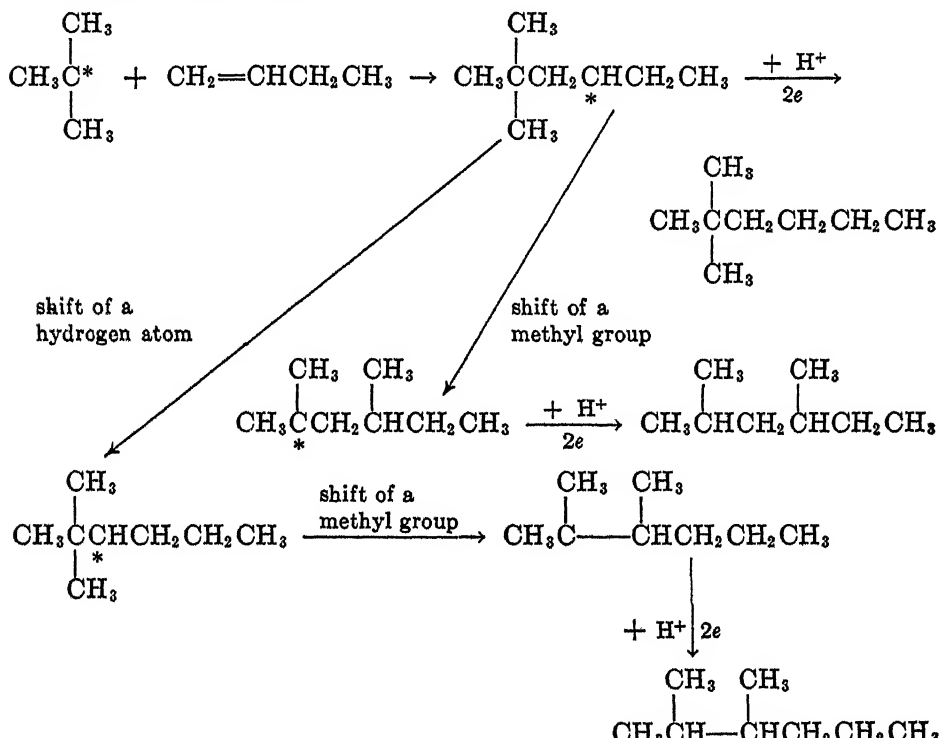
2. Reaction with propene:



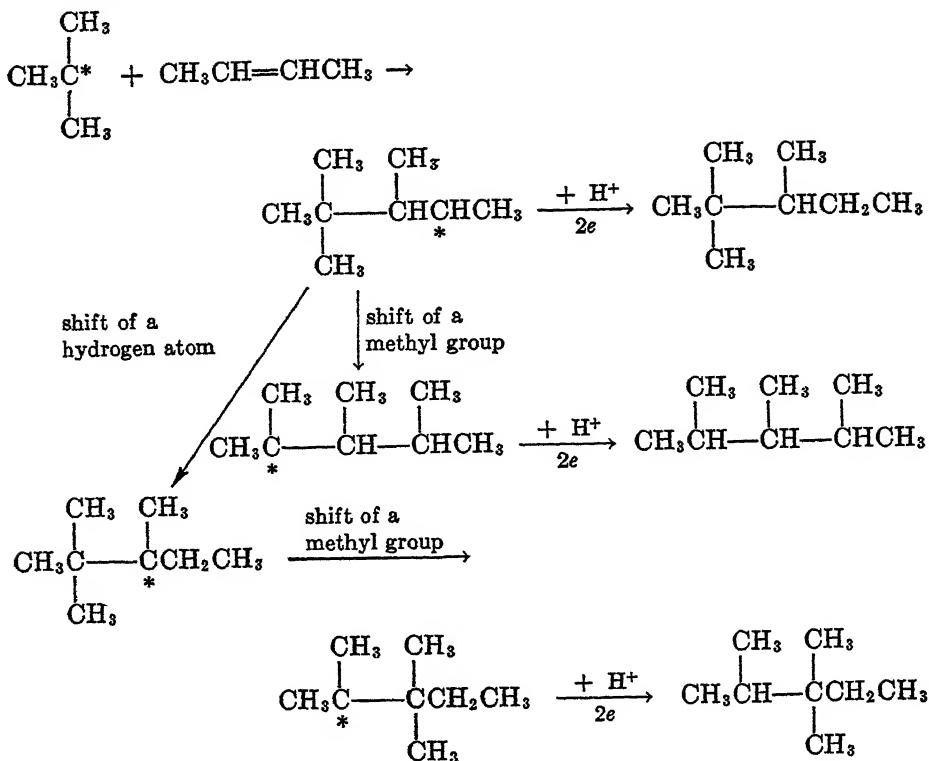
3. Reaction with isobutene:



4. Reaction with 1-butene:



5. Reaction with 2-butene:



The predicted and experimentally observed products in the alkylation of isobutane by ethene, propene, and "butenes" are compared in table 38.

Table 38 indicates a close parallelism between predicted and observed products. Unfortunately, no details were given about the catalysts used with the enumerated reactants. The predicted primary products for 1-butene, 2-butene, and isobutene were grouped together, "since the experimental data show that the hydrocarbons obtained using either normal butenes or isobutenes are very similar." This behavior is presumably caused by isomerization of the "butenes" prior to reaction with isobutane (37), although "depolymerization" of carbonium ions was taken to be "probably one of the chief causes for the complexity of the products found in all alkylation reactions."

Ciappetta supported his assigned mechanism of alkylation by a literature survey of several reactions of alkanes, alkenes, and alkanols in the presence of sulfuric acid: alkylation of isobutane; hydrogen exchange of alkanes in deuterio-sulfuric acid; degradation of alkanes (reversal of alkylation); polymerization of alkenes; depolymerization of alkenes; conjunct polymerization of alkenes; hydrogen exchange of alkenes in deuteriosulfuric acid; isomerization of alkenes; and dehydration of alkanols. The physical, protolytic, and hydrogenation-

dehydrogenation properties of sulfuric acid were considered also. Its marked hydrogenation-dehydrogenation properties were ascribed to a high dielectric constant, which would also enable it to ionize alkanes. This ionizing ability of sulfuric acid is in agreement with the large autoprotolysis constant,

$$K = [\text{H}_3\text{SO}_4^+][\text{HSO}_4^-]$$

for the reaction



It was pointed out that alkanes without a tertiary hydrogen atom cannot ionize to any great extent in sulfuric acid. The reaction conditions for satisfactory alkylation of alkanes by alkenes were listed as follows (14):

TABLE 38

Comparison of the primary compounds predicted by the carbonium-ion mechanism with those found experimentally in the alkylation of isobutane

(Ciapetta (14))

REACTANTS	PREDICTED PRODUCTS	FOUND EXPERIMENTALLY
Isobutane + ethene.....	2,2-Dimethylbutane 2,3-Dimethylbutane 2-Methylpentane	2,2-Dimethylbutane 2,3-Dimethylbutane 2-Methylpentane
Isobutane + propene	2,2-Dimethylpentane 2,3-Dimethylpentane 2,4-Dimethylpentane	2,2-Dimethylpentane 2,3-Dimethylpentane 2,4-Dimethylpentane
Isobutane + "butenes".....	2,2,4-Trimethylpentane 2,3,4-Trimethylpentane 2,2,3-Trimethylpentane 2,3,3-Trimethylpentane 2,2-Dimethylhexane 2,4-Dimethylhexane 2,3-Dimethylhexane	2,2,4-Trimethylpentane 2,3,4-Trimethylpentane 2,2,3-Trimethylpentane 2,3,3-Trimethylpentane (Not reported) 2,4-Dimethylhexane 2,3-Dimethylhexane 2,5-Dimethylhexane

"1. Only those paraffins which possess a tertiary hydrogen atom will take part in the reaction. To date no one has observed that normal paraffins react with olefins in the presence of concentrated sulfuric acid. This explains why McAllister [37] was unable to react neopentane or neohexane with olefins, which according to his mechanism should give carbon to carbon fission as does isobutane, isopentane and isohexane. Without a tertiary hydrogen atom ionization cannot take place in sulfuric acid to any great extent.

"2. The success of the alkylation reaction will depend on the ratio of isoparaffin to olefin. In the initial step of the proposed mechanism, the formation of a carbonium-ion by the isoparaffin, the forward reactions will be influenced in two ways by the addition of olefin to the isoparaffin in concentrated sulfuric acid. If the olefin is added in small quantities it immediately reacts with the carbonium-ion formed from the isoparaffin and shifts the equilibrium to the right in both steps with the result that the isoparaffin will play a substantial rôle in the reaction. However, if the olefin is added in large amounts so that its concentration far exceeds that of the carbonium-ion formed from the isoparaffin, it can also form

a positive ion as postulated by Whitmore. Increasing the concentration of the positive ion will shift the equilibrium to the left and result in a poor conversion of the isoparaffin.

"3. The concentration of the acid must be higher than 87% in order to get complete saturation of the products since acids of lower strengths as found by Ipatieff [31] give olefins in the product. The hydrogenation-dehydrogenation properties of sulfuric acid depend on its concentration.

"4. A large volume of sulfuric acid must be used for the best results. Ipatieff [31] found in conjunct polymerization of olefins that decreasing the ratio of sulfuric acid to hydrocarbon below 1 to 1 by weight results not only in the formation of large amounts of olefins in the fraction boiling below 225°C., but also decreases the yield of hydrocarbons boiling in this range.

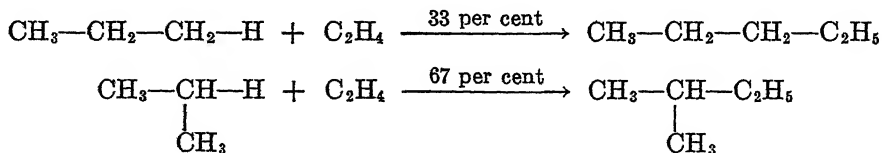
"5. Since the isoparaffin is only slightly soluble in the acid, the probability that the olefin will react with the positive fragment from the isoparaffin will be directly proportional to the efficiency of the agitation. Unless the olefin can come in the vicinity of the carbonium ion formed from the isoparaffin, it will form its own ion and combine with more olefin, and consequently low yields of alkylate will be obtained. This fact was recognized early in the development of the alkylation process, when it was found that effective dispersion of the hydrocarbons in the acid is essential to obtain the best yields of product.

"6. Since concentrated sulfuric acid is a hydrogenation-dehydrogenation agent, the presence of diolefins in the olefin feed should have no effect on the character of the alkylate since these are readily hydrogenated to olefins. The presence of butadiene in concentrations as high as 22% [7, 36] caused no change in the composition or yield of alkylate. The only observed effect was the more rapid deterioration of the catalyst due to the dehydrogenation of part of the unsaturated hydrocarbon to form cyclic olefins as in conjunct polymerization."

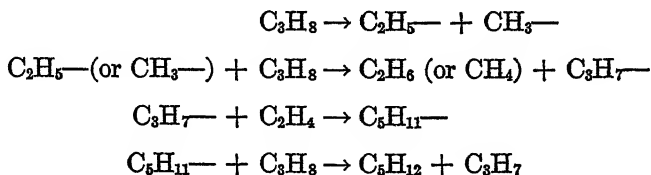
No single mechanism so far proposed answers adequately all questions regarding the mechanism of alkylation of alkanes by alkenes or alkyl halides. Much more experimental work will be required to clarify the mechanism of these reactions and to give the reasons for formation of specific products. A theory for the future development of alkylation of alkanes should account kinetically and quantitatively for all reaction products. It must allow for the effect of various side reactions, including isomerization, hydrogenation-dehydrogenation, polymerization-depolymerization, and even dealkylation. In particular, all of the theories presented fail to correlate adequately the structure and chemical reactivity of hydrocarbons and related esters. Considerations of inter- and intramolecular forces are conspicuously lacking.

B. THERMAL ALKYLATION

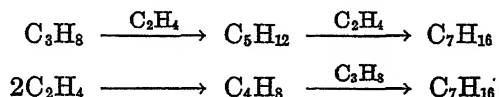
Frey and Hepp concluded that, in thermal alkylation with ethene, replacement of the secondary hydrogen atom of propane by ethyl proceeds more rapidly than substitution of the primary hydrogen atom by ethyl (18):



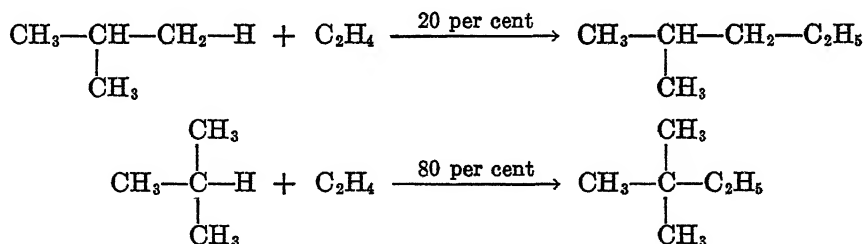
The propane alkylation mechanism was assumed to be a chain reaction, as in the thermal decomposition of alkanes (45). Propyl radicals were assigned the rôle of chain carriers:



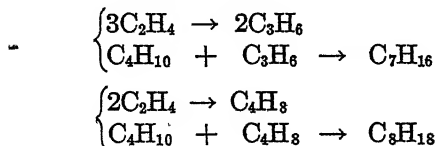
The presence of hexanes in the product from propane and ethene was ascribed in part to a reaction between propane and propene, which was stated to form substantial amounts of 2,3-dimethylbutane and 2-methylpentane under non-catalytic conditions. Heptanes were also present, indicating a further ethylation of the pentane(s) or a dimerization of ethene into butenes, which then alkylated a part of the propane feed:



The expected order of hydrogen replacement in the thermal ethylation of alkanes was given as tertiary hydrogen > secondary hydrogen > primary hydrogen. Replacement of the tertiary hydrogen atom of isobutane by ethyl was actually observed to exceed that of the primary hydrogen atoms of isobutane by ethyl:



The presence of heptanes and octanes in the product from isobutane and ethene was ascribed to probable alkylations by propene and butenes:



Schmerling (50) has pointed out that the difference between catalytic and thermal alkylation of isobutane with ethene can be explained by assuming that, as has already been discussed, the primary product in the catalytic reaction is an

ester, the carbon skeleton of which rearranges during its conversion into alkane, whereas the primary product of thermal alkylation is an alkane which does not isomerize under the reaction conditions.

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THE CHEMISTRY OF OXINDOLE

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CONTENTS

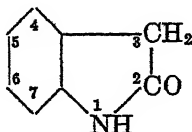
I. Introduction.....	443
II. Synthesis of oxindole and of oxindole derivatives.....	443
III. General properties of oxindole	
A. Physical properties.....	452
B. Salts.....	453
C. Tautomerism.....	453
D. Oxidation and reduction.....	454
E. Acyl and alkyl derivatives of oxindole.....	454
F. 3,3-Diaryl derivatives of oxindole.....	460
G. Halogenation, nitration, and sulfonation.....	460
H. Condensation with aldehydes and ketones.....	466
I. Amino derivatives of oxindole.....	467
J. Hydroxy derivatives of oxindole.....	470
IV. References.....	475

I. INTRODUCTION

In 1866 and 1868 Baeyer (4, 10) published the results of his researches on the reduction of isatin. In addition to isatide, which had been obtained previously by Laurent (128, 130, 131) and by Erdmann (49), Baeyer obtained dioxindole, $C_8H_7NO_2$, by the further reduction of which oxindole, C_8H_7NO , was prepared.

Baeyer (5) established the constitution of oxindole as the lactam of 2-amino-phenylacetic acid through its synthesis by the reduction of 2-nitrophenylacetic acid with tin and hydrochloric acid.

Current practice in oxindole nomenclature is to number the positions as shown in the formula below. Other systems of numbering have been used at times by



some workers (28, 29, 30, 31, 33, 169), but the system shown here is employed generally at the present time. In the English and German literature oxindole is frequently called indolinone.

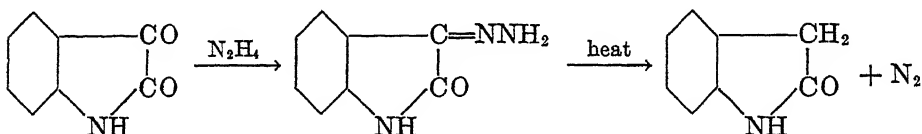
II. SYNTHESIS OF OXINDOLE AND OF OXINDOLE DERIVATIVES

Baeyer and Knop (10) found that when isatin is reduced with sodium amalgam in alkaline medium 3-hydroxyoxindole (dioxindole) is obtained. Further reduction of dioxindole with tin and mineral acids or by sodium amalgam in acid medium gave oxindole. One convenient procedure for preparing oxindole is that of

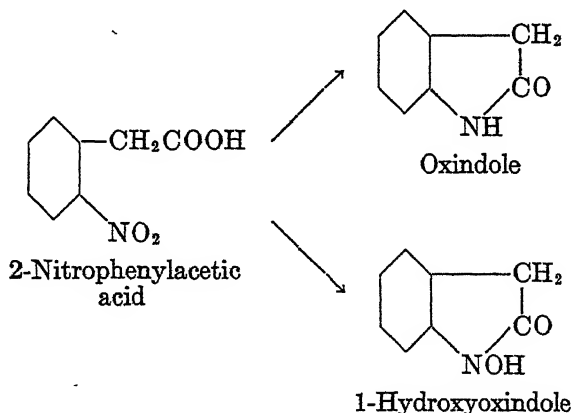
Marschalk (141, 142). In this procedure isatin is reduced to dioxindole through the agency of sodium hydrosulfite. Dioxindole is then reduced to oxindole by the action of sodium amalgam in a solution saturated with carbon dioxide. The reduction of a number of substituted dioxindoles to the corresponding derivatives of oxindole has been accomplished by Wahl and coworkers (204, 205).

v. Braun and Hahn (26) prepared dioxindole-4-carboxylic acid by reducing isatin-4-carboxylic acid with sodium amalgam. Dioxindole-4-carboxylic acid undergoes disproportionation when heated in alcohol solution, yielding oxindole-4-carboxylic acid and isatin-4-carboxylic acid in equivalent quantities. Oxindole-4-carboxylic acid can also be prepared by reducing dioxindole-4-carboxylic acid with sodium amalgam under proper conditions.

Isatin was also reduced to oxindole through the agency of hydrazine by Curtius and Thun (43).



The first synthesis of oxindole (other than by the reduction of isatin) and the one which established its constitution with certainty was by Baeyer (5) through the reduction of 2-nitrophenylacetic acid with tin and hydrochloric acid.



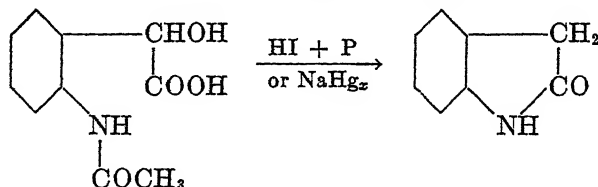
Reduction of 2-nitrophenylacetic acid with zinc and hydrochloric acid gives both oxindole and 1-hydroxyoxindole (also sometimes called 1,2-dioxindole) (163, 164, 165).

Substituted oxindoles have been prepared by the reduction of substituted derivatives of 2-nitrophenylacetic acid by Wispec (212), Smith and MacMullen (172), Wahl and Livovschi (138, 139, 207), Ruggli and Grand (166), Parks and Aldis (158), Wahl and Bagard (200), Hahn and Schulz (68), Hahn and Tulus (69), Trinius (196), Heller (78), and Gabriel and Meyer (56). König and Reissert (114) obtained oxindole and *o*-aminophenylacetanilide by the reduction of *o*-nitrophenylacetanilide.

Heller (77) found that reduction of *N*-acetoxyoxindole with zinc dust and acetic acid gave oxindole.

Di Carlo (44) found that catalytic reduction of *o*-nitrophenylacetic acid gave oxindole. Under certain conditions some 1-hydroxyoxindole was obtained as a by-product. The procedure given by Di Carlo seems to offer a convenient method for the synthesis of oxindole. Koelsch (113) has utilized a similar procedure for the synthesis of 5-methoxyoxindole.

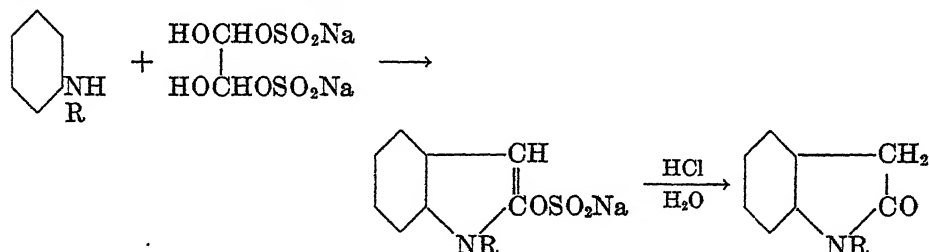
Oxindole was also prepared by Suida (183) through the reduction of 2-acetaminomandelic acid by either hydriodic acid and phosphorus or sodium amalgam.



Baeyer and Comstock (9) prepared oxindole from the barium salt of 2-aminophenylacetic acid by acidifying and then heating.

Pschorr and Hoppe (161) prepared oxindole from 2-aminobenzyl cyanide by treatment with aqueous sodium hydroxide and subsequent acidification.

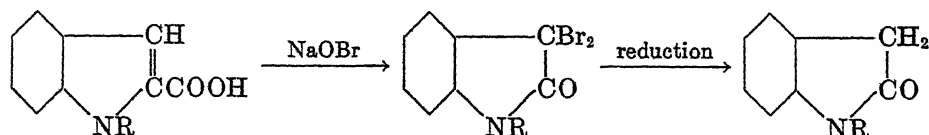
A procedure developed by Hinsberg (87, 88) serves for the preparation of *N*-alkyloxindoles. A secondary aromatic amine is condensed with the sodium



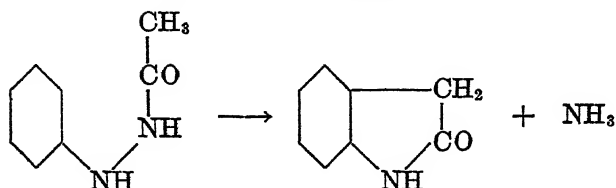
bisulfite addition compound of glyoxal. The resulting product gives an *N*-alkyloxindole on treatment with aqueous hydrochloric acid.

Oxindole was obtained by Mazzaro and Borgo (150) by steam distillation in the presence of hydrochloric acid of the product obtained when indole is treated with sulfur chloride.

N-Alkylindoles and *N*-substituted indole- α -carboxylic acids have been converted into the corresponding oxindoles by Colman (40) and by Michaelis (152). The *N*-alkylindole- α -carboxylic acid (or *N*-alkylindole) is treated with sodium hypobromite, giving a 1-alkyl-3,3-dibromo-oxindole which on reduction gives the corresponding 1-alkyloxindole.

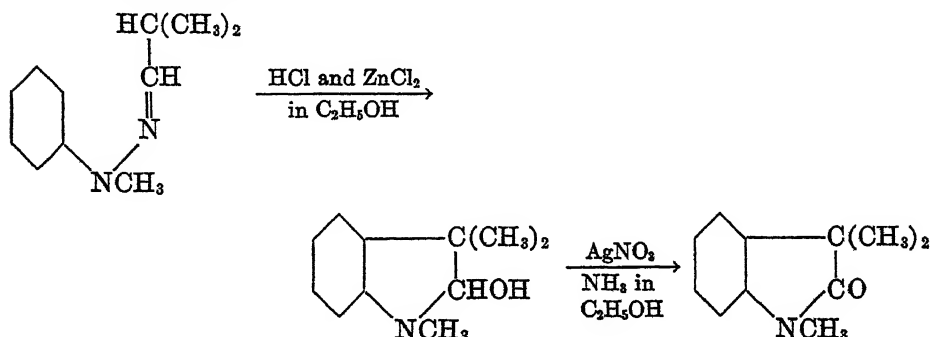


Brunner (29, 30, 33) prepared oxindole by heating β -acetylphenylhydrazine with lime at 200–220°C. This procedure has been extended by Brunner (34, 35,

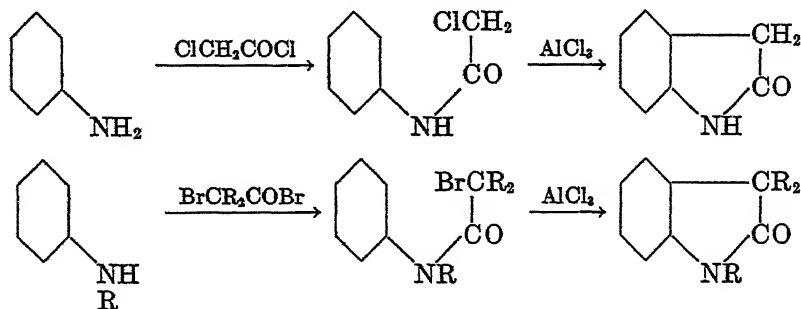


36) and by others (59, 60, 169, 195, 199) to the preparation of many substituted oxindoles, especially 3,3-dialkyl derivatives of oxindole.

A similar preparation of substituted oxindoles also due to Brunner (28, 31) is shown in the following scheme:

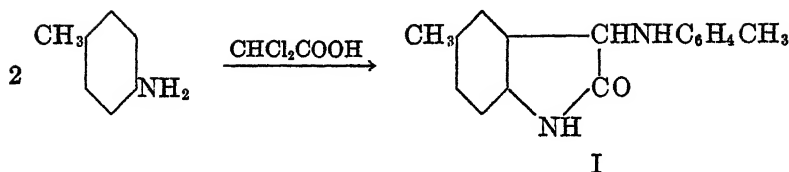


Another convenient and general method for the preparation of oxindole and of *N*-substituted oxindoles is that of Stollé (62, 63, 179, 180, 182). An α -halogenated acid chloride or bromide is condensed with an aromatic amine. Subsequent ring closure with aluminum chloride yields the corresponding oxindole.



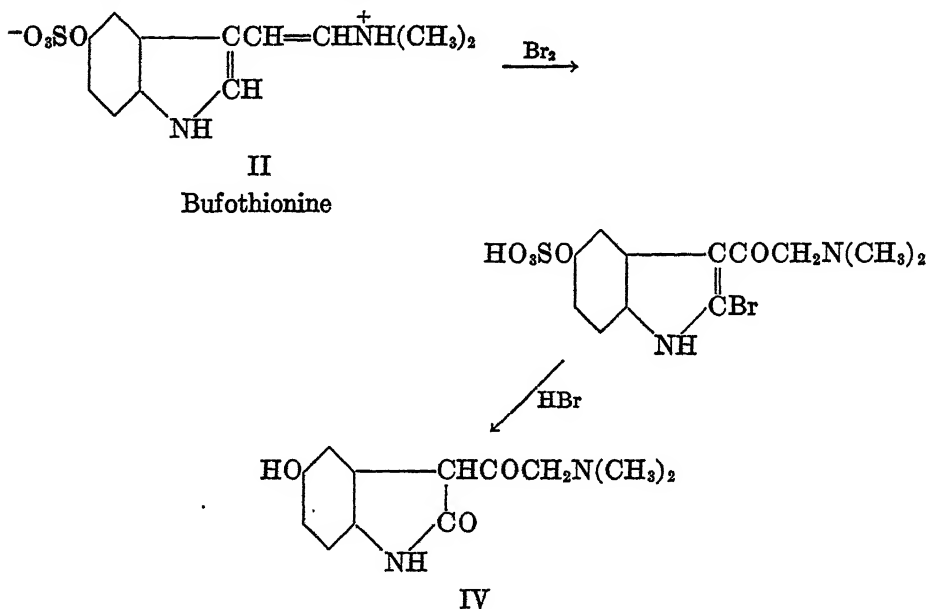
The Stollé synthesis has also been utilized by a number of other investigators (98, 100, 107, 138, 139, 160, 207). Stollé found that *N*-benzylchloroacetanilide on treatment with aluminum chloride gave oxindole with the splitting out of the benzyl group (180).

Oxindole derivatives of the type of I are obtained from arylamines and

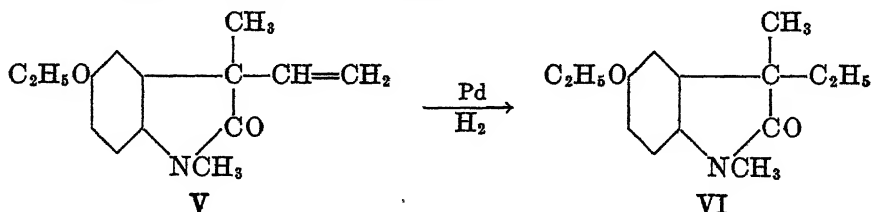


dichloroacetic acid in a reaction discovered by P. J. Meyer (151) and subsequently studied by Duisberg (46), Heller (79, 80), and Paucksch (159).

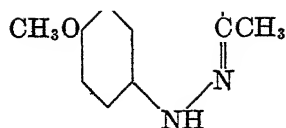
Wieland and Wieland (210) obtained the oxindole derivative IV from bufothi-one (II) in the manner shown below:



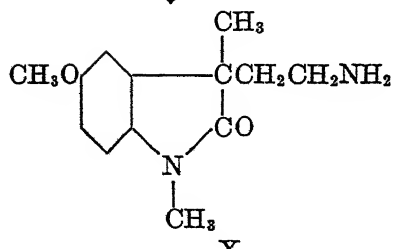
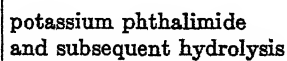
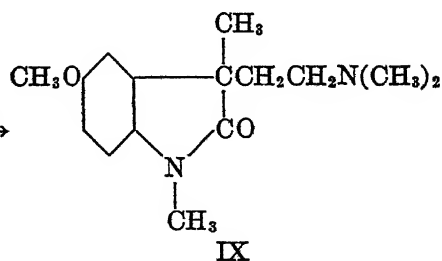
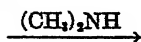
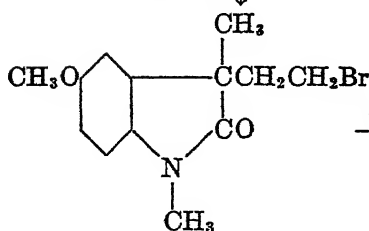
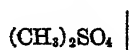
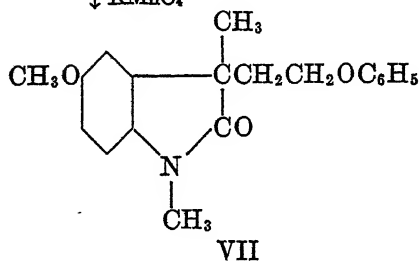
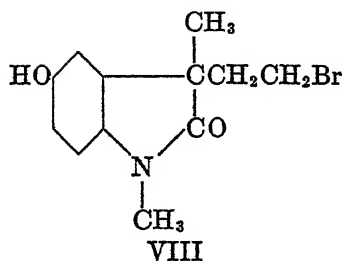
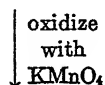
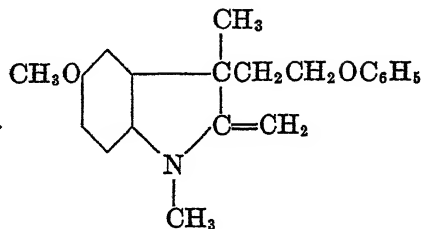
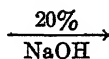
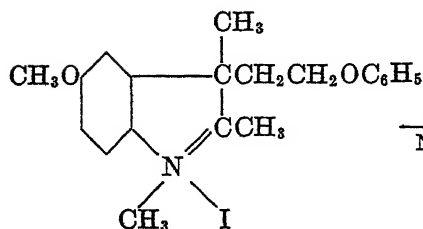
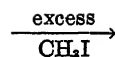
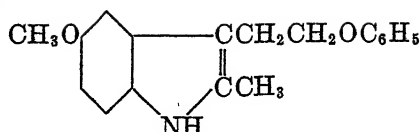
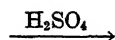
Stedman and Barger (173), in the course of the investigation of the structure of physostigmine (eserine), obtained the oxindole derivative V as a degradation product. Catalytic reduction of V yielded VI.



In a series of papers pointing to the synthesis of physostigmine, Robinson, Boyd-Barrett, and King (23, 24, 108, 109) developed syntheses of some interesting oxindole derivatives (VII, VIII, IX, X).

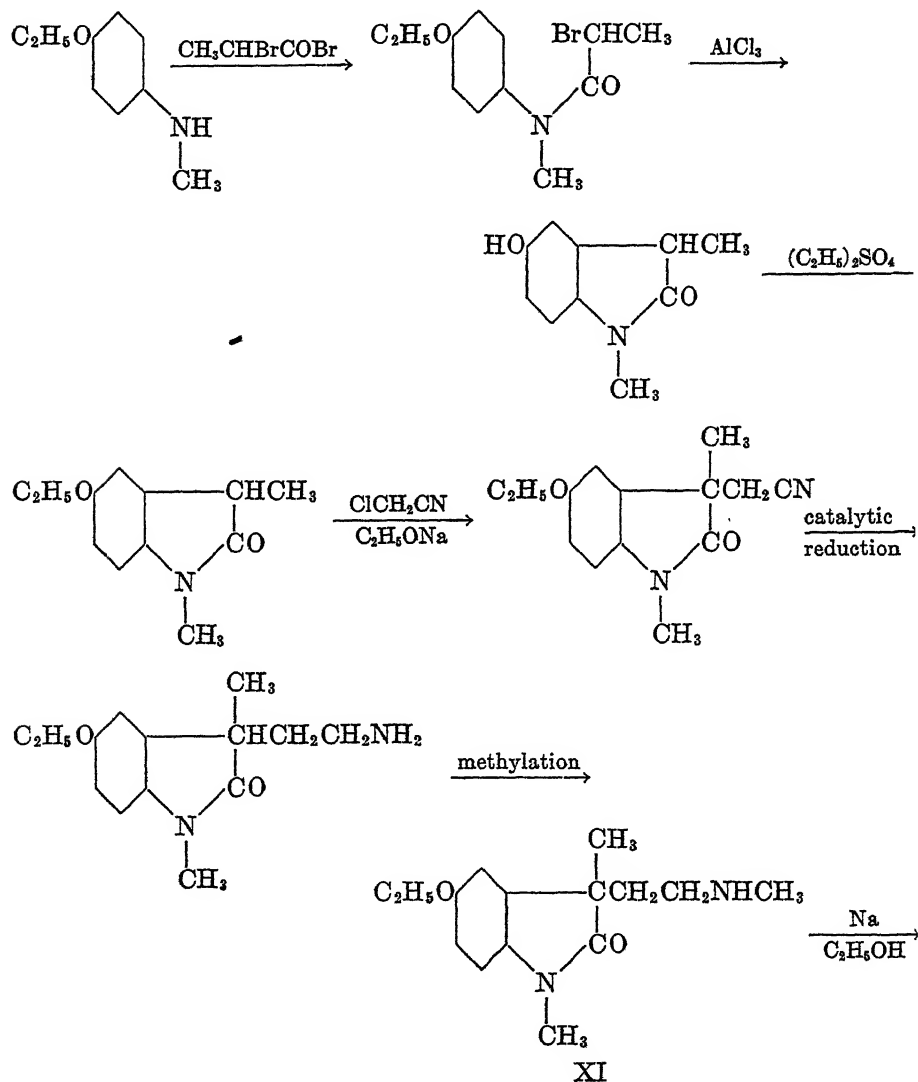


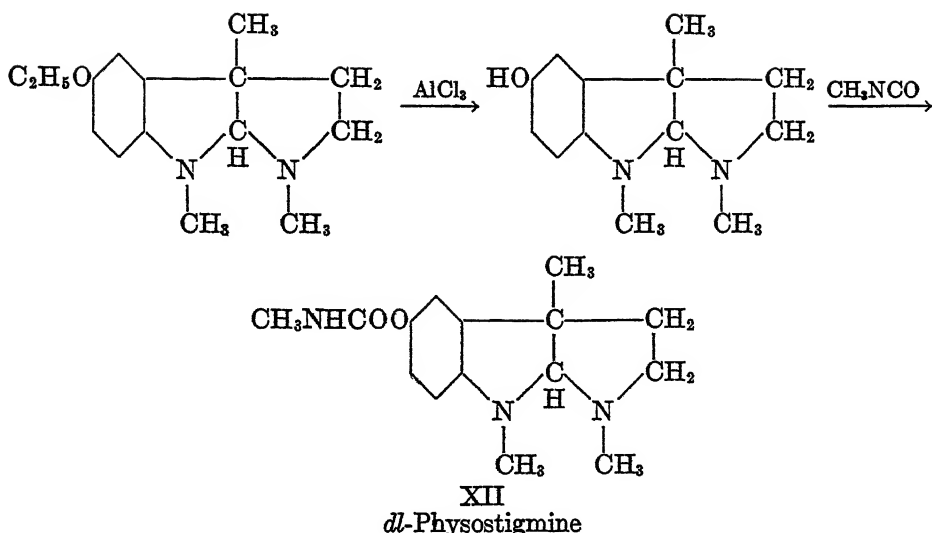
γ -Phenoxypropylacetone
p-methoxyphenylhydrazone



V

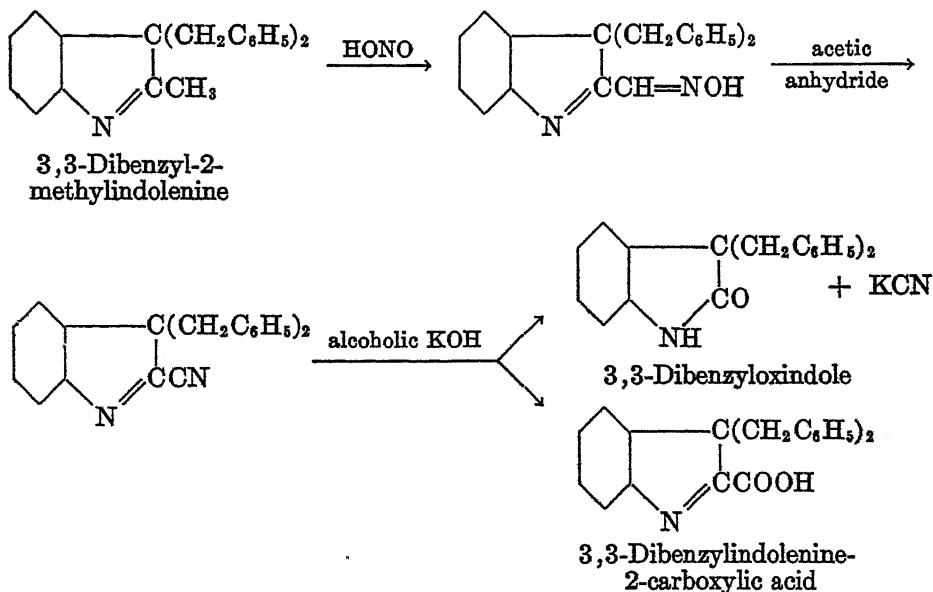
The total synthesis of physostigmine was accomplished by Julian and coworkers (98, 99) in a research involving some beautiful oxindole chemistry. The synthesis follows in outline:



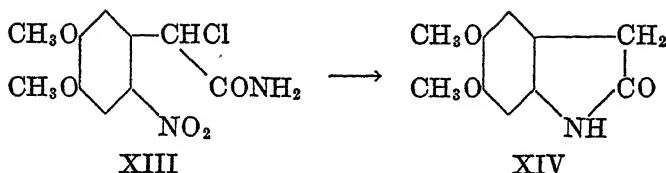


Resolution of racemic XI and continuation of the synthesis with *l*-XI gave *l*-physostigmine, identical with the natural product.

Leuchs and Overberg (133) prepared 3,3-dibenzylloxindole from 3,3-dibenzyl-2-methylindolenine, as outlined below:

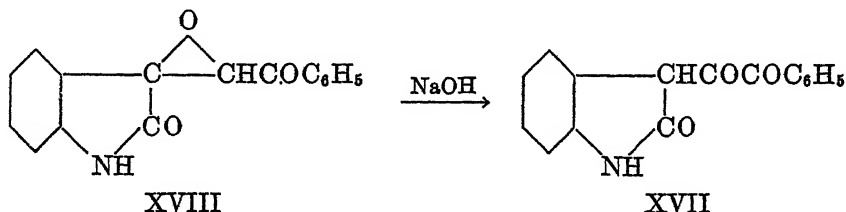


Hahn and Tulus (69) prepared several oxindole derivatives by the catalytic reduction of certain α -chloro- α -(*o*-nitroalkoxyphenyl)acetanilides. The reduction of XIII yielded 5,6-dimethoxyoxindole (XIV).

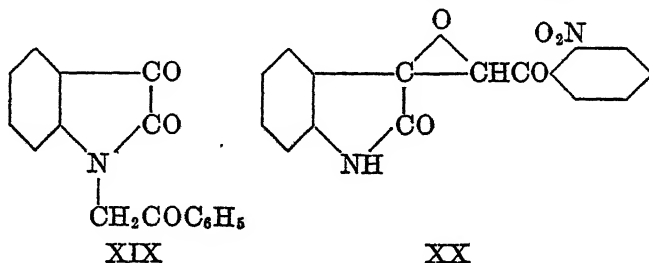


5,6-Methylenedioxyoxindole (XV) and 5-acetoxy-6-methoxyoxindole (XVI) were prepared in similar fashion. Under different catalytic conditions the reduction of XIII gives the hydrochloride of 2-amino-3,4-dimethoxyphenylacetamide as well as the dimethoxyoxindole (XIV).

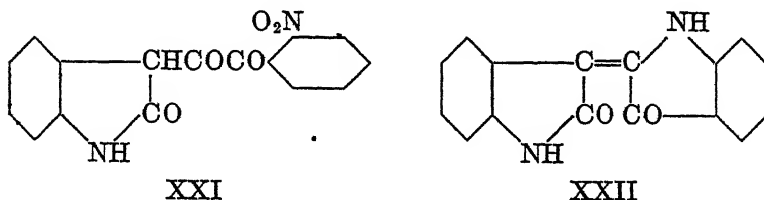
Ainley and Robinson (2) found that 3-benzoylformyloxindole (XVII) is formed when isatylideneacetophenone oxide (XVIII) is treated with alkali. The latter compound (XVIII) is formed when the sodium salt of isatin is treated with



phenacyl bromide. Ainley and Robinson had expected that 1-phenacylisatin (XIX) would be the product in this reaction but found that XVIII was the actual substance obtained. In support of the structures assigned to this substance (XVIII) and to the rearrangement product (XVII), Ainley and Robinson found

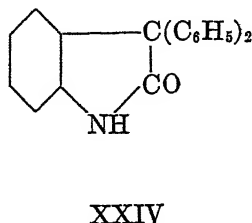
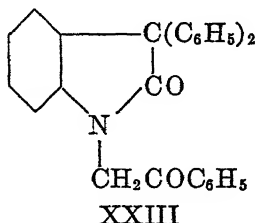


that isatylidene-*o*-nitroacetophenone oxide (XX) and *o*-nitrobenzoylformyloxindole (XXI) both yield indirubin (XXII) on reduction.

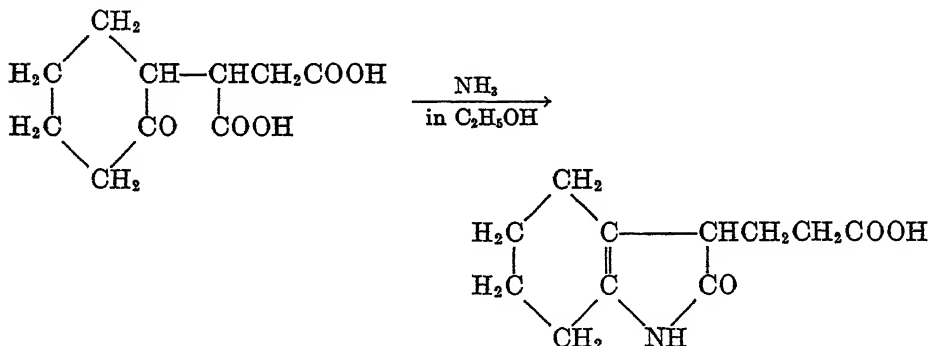


Measurements of the absorption spectra of benzoylformyloxindole (XVII) have been reported by Bergstrom and Robinson (18).

A recent attempt (189) to prepare 1-phenacylisatin from isatin-1-acetyl chloride and benzene through the agency of aluminum chloride led to the preparation of 3,3-diphenyl-1-phenacyloxindole (XXIII) and not XIX as expected. This condensation with two molecules of benzene in the 3-position seems to be general in the isatin series, since 3,3-diphenyloxindole (XXIV) was obtained from isatin, benzene, and aluminum chloride (189).



An interesting preparation of 4,5,6,7-tetrahydrooxindole-3-propionic acid from 2-ketocyclohexane- α -glutaric acid has been described by Kendall and co-workers (105, 106).



A number of brominated and iodinated derivatives of oxindole-3-propionic acid have been described by these workers. The structures suggested for several of these derivatives are somewhat unorthodox and, being supported by evidence which appears quite inadequate, must be regarded as far from established. The melting point given for their oxindole-3-propionic acid is quite different from that given elsewhere (64) for oxindole-3-propionic acid prepared by more conventional methods.

III. GENERAL PROPERTIES OF OXINDOLE

A. Physical properties

Oxindole crystallizes from water in colorless needles melting at 126–127°C. The substance boils at 195°C. at 17 mm. (202) and at 227°C. at 73 mm. (43). It is soluble in hot water, alcohol, benzene, ether, and acetic acid. It is more soluble in alkaline solutions than in water. The heat of combustion at constant volume has been found to be about 950.5 kg.-cal. per mole (19).

B. Salts

Oxindole forms a white silver salt, C_8H_6ONAg , on treatment of its aqueous solution with cold ammoniacal silver nitrate solution (10). On prolonged heating the silver nitrate is reduced by the oxindole.

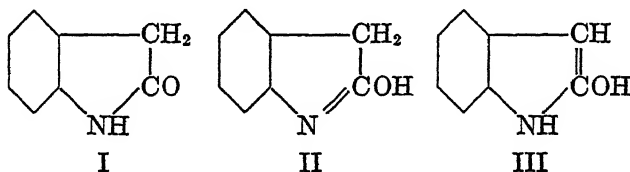
The sodium salt of oxindole is obtained from oxindole and sodium amalgam in warm benzene (209). This salt is also obtained by the treatment of oxindole with sodium ethoxide (78).

Heating oxindole with barium hydroxide solution at $150^{\circ}C$. gives the barium salt of 2-aminophenylacetic acid. The latter salt on acidification again yields oxindole (9, 141, 142).

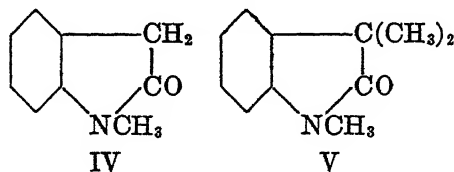
Oxindole combines with hydrochloric acid to give a hydrochloride which is easily soluble in water (10).

C. Tautomerism

Oxindole is usually regarded as the lactam (I) of *o*-aminophenylacetic acid. However, the lactim (II) and the enol (III) formulas represent possible structures.



Ramart-Lucas and Biquard (162) found the absorption spectra of oxindole to be quite similar to those of *N*-methyloxindole (IV) and 1,3,3-trimethyloxindole (V); since the latter compound can exist only in the lactam form, they consider

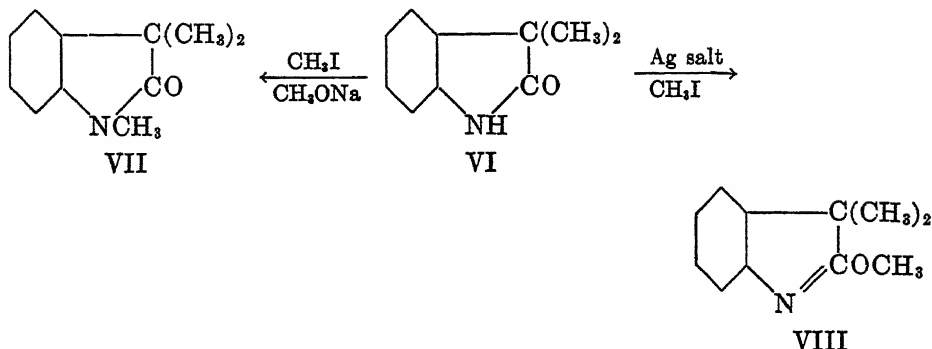


that I is probably the correct structure for oxindole.

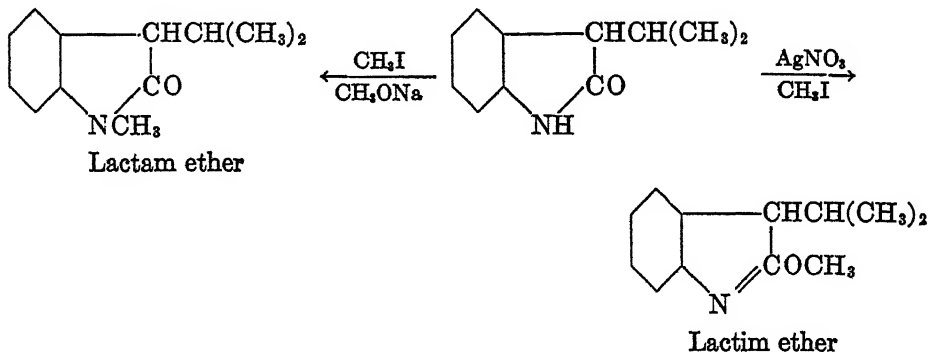
Julian (101) writes oxindole as the lactam (I) but found that in the Grignard machine two moles of reagent are consumed and two molecules of gas liberated. He feels that this indicates enolization of oxindole (I) in the sense represented by formula III rather than formula II. The reaction of 1-ethyloxindole with the Grignard reagent has been studied by Stollé (182).

Alkylation of oxindole with alkyl halides and sodium ethoxide gives the corresponding *N*-alkyloxindole (9). *O*-Alkyl ethers corresponding to the *O*-alkyl isatin derivatives have not been prepared from oxindole itself. The lactam and lactim ethers of certain 3,3-dialkyloxindoles have been prepared, however. Thus, Brunner (29) prepared 1,3,3-trimethyloxindole (VII) from 3,3-dimethyloxindole (VI) through the agency of methyl iodide and sodium methoxide, while

the lactim ether VIII resulted when the silver salt of VI was treated with methyl iodide.



Schwarz (16) likewise obtained lactam and lactim ethers from 3-isopropylloxindole and methyl iodide.



D. Oxidation and reduction

Oxindole gives indole when its vapor is passed over hot zinc (7). On prolonged heating with ammoniacal silver nitrate solution oxindole reduces the silver nitrate to a mirror (10).

On prolonged contact with air an aqueous solution of oxindole is oxidized in part, yielding dioxindole (10).

Reduction of 1,3,3-trimethyloxindole with sodium and alcohol gives 1,3,3-trimethylindolinol-2 (28, 36).

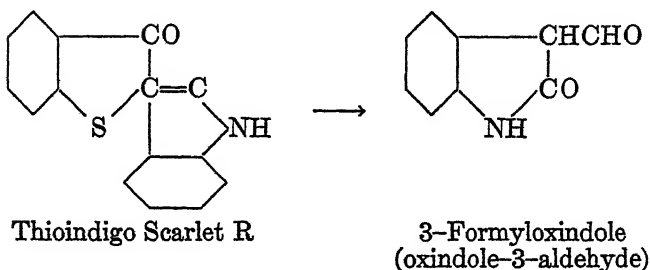
E. Acyl and alkyl derivatives of oxindole

Suida (184) prepared 1-acetyloxindole by the action of acetic anhydride on oxindole. 3-Methyloxindole and other oxindole derivatives similarly yield 1-acetyl derivatives (29, 30, 150, 165, 169, 199).

Treatment of the sodium salt of oxindole with one molecular proportion of benzoyl chloride yields 1-benzoyloxindole, while with excess benzoyl chloride 1,3,3-tribenzoyloxindole is formed (78).

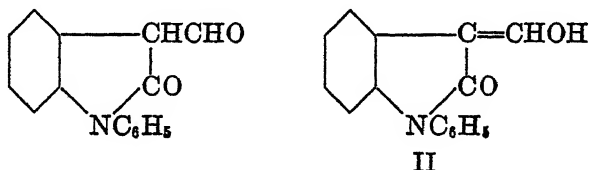
3-Formyloxindole (oxindole-3-aldehyde) was first prepared by Friedlander and

coworkers (53, 54) by treatment of Thioindigo Scarlet R with alcoholic sodium hydroxide.

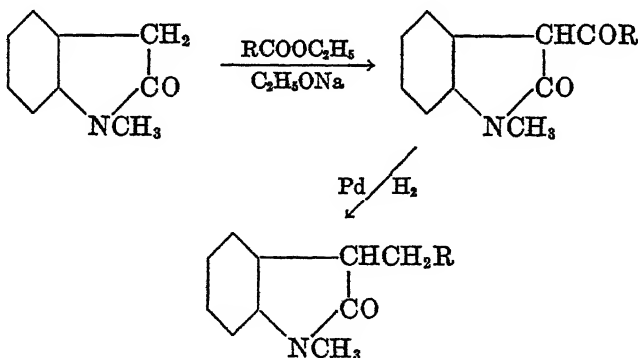


Friedlander also prepared the *N*-methyl analog from *N*-methyl Thioindigo Scarlet R. The procedure has also been employed by Kalb and Berrer (104) for the preparation of 3-formyl-5,7-diiodoöxindole.

In 1932 Stollé, Hecht, and Becker prepared 3-formyl-1-phenyloxindole (I) through the condensation of ethyl formate with 1-phenyloxindole. They formulated the compound as the tautomeric structure II.



The reaction was developed independently by Julian (100) in 1934 and used for the preparation not only of 3-formyloxindoles but also of 3-acyloxindoles in general (101).

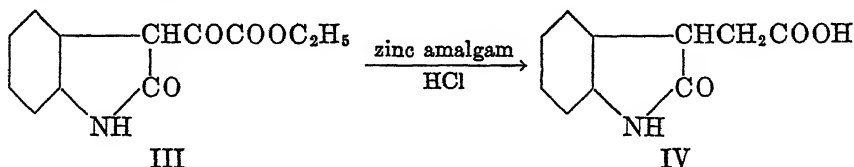


Catalytic reduction of the latter compounds affords a new synthesis of 1,3-dialkyloxindoles. On the other hand, Horner (89) has reported that 3-acyloxindoles without substituent groups on nitrogen could not be reduced to 3-alkyl derivatives.

This synthesis of 3-acyloxindoles was applied by Julian only in the case of the *N*-alkyl derivatives. Horner (89) extended the reaction to include the condensation of oxindole itself with various esters, as shown in the accompanying chart. The condensation of *N*-substituted oxindoles with esters has also been studied by

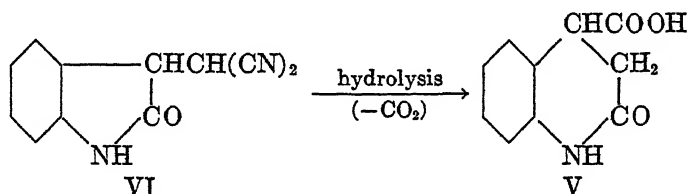
A similar reaction between isatin (position 1) and acrylonitrile has recently been reported by Di Carlo and Lindwall (45).

The reduction of ethyl oxindole-3-glyoxalate (III) under Clemmensen conditions has been studied by Horner (89). Reduction of III with zinc amalgam and hydrochloric acid gave a product melting at 217°C., which Horner described as oxindoleacetic acid (IV).

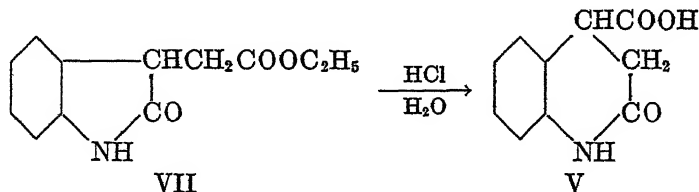


This reduction has been reinvestigated by Sumpter, Miller, and Hendrick (192), who found that Horner's "oxindoleacetic acid", like the "oxindoleacetic acid" of Gränacher (57, 58), is in reality 2-keto-1,2,3,4-tetrahydroquinoline-4-carboxylic acid (V) (1, 82, 83).

The formation of V in the reduction and hydrolysis of III is quite in keeping with the results of Zrike and Lindwall (213), who obtained V in the hydrolysis of VI.



Horner (89) also reported that the reduction of III gave the ethyl ester of oxindoleacetic acid (VII) when zinc amalgam and acetic acid were employed. This has been confirmed by Sumpter, Miller, and Hendrick (192), who further found that hydrolysis of VII yielded V.



It has been stated (143) that the Clemmensen reduction of α -keto acids and esters always gives the corresponding α -hydroxy acid or ester rather than the completely reduced acid. Obviously the preparation of V and of VII through the Clemmensen reduction of III constitutes an exception to this rule.

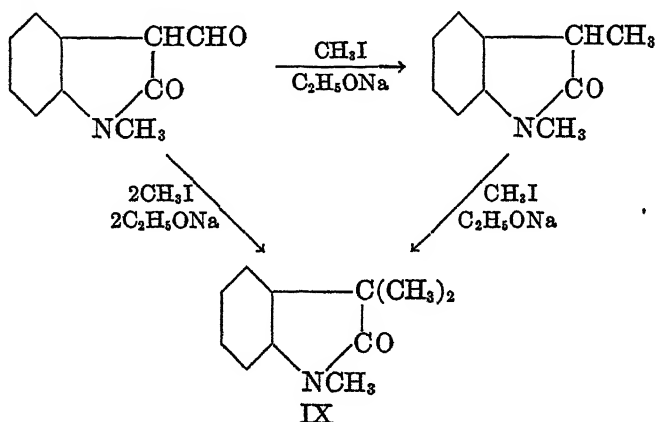
Alkyl derivatives of oxindole have been prepared by many workers (23, 24, 28, 29, 30, 31, 33, 34, 35, 36, 40, 62, 63, 79, 80, 87, 88, 107, 108, 109, 133, 134, 138, 139, 152, 173, 179, 180, 182, 202) and by a variety of procedures. A number of these general methods have already been discussed in section II of this paper.

Direct alkylation of oxindole and of *N*-alkyloxindoles has been accomplished

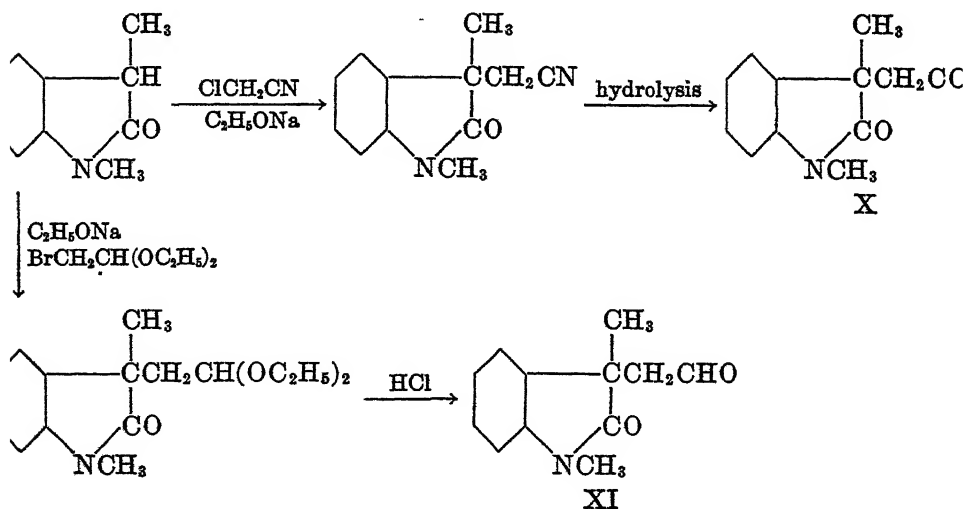
by Brunner (30) and by Julian (98, 100, 101). Julian seemingly overlooked the work of Brunner, for he stated, "The literature records no efforts at direct alkylation of oxindoles." On the contrary, Brunner (30) had recorded the alkylation of 3-methyloxindole by the action of methyl iodide.



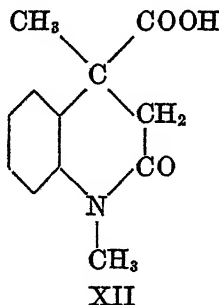
Julian found that IX was formed when 1-methyl-3-formyloxindole was methylated by the action of methyl iodide and sodium ethoxide.



This same technique has been applied by Julian in the synthesis of 1,3-dialkyloxindole-3-acetic acid derivatives (X) and derivatives of 1,3-dialkyloxindole-3-acetaldehyde (XI).

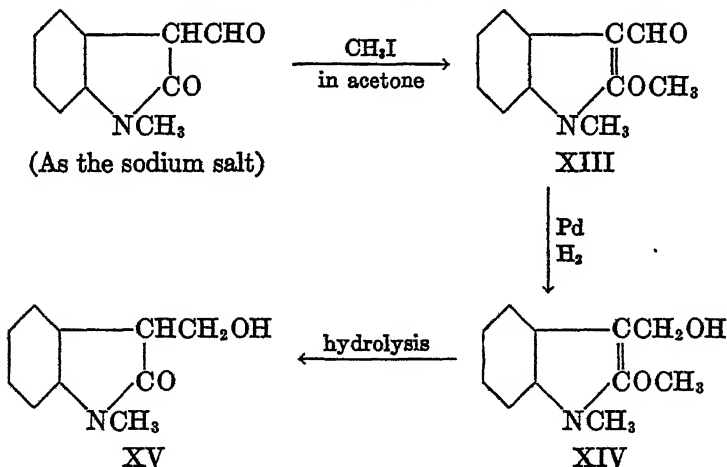


In view of the non-existence of "oxindoleacetic acid" (1, 57, 58, 83, 192), it seems entirely possible that Julian's X has been incorrectly formulated and that the compound may be the quinoline derivative XII. This point has not been investigated.



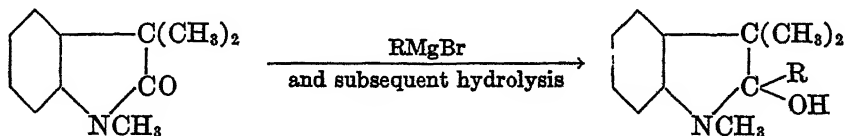
Julian was unable to effect alkylation in position 3 in the case of oxindole itself or of 1-methyloxindole. Apparently it is necessary that there be either an alkyl or an acyl group in position 3 for further alkylation in this position to be accomplished.

Treatment of the sodium salt of 1-methyl-3-formyloxindole with methyl iodide in acetone results in the formation of the *O*-methyl derivative (XIII) (101). Reduction of this *O*-methyl ether gives the alcohol (XIV), which on

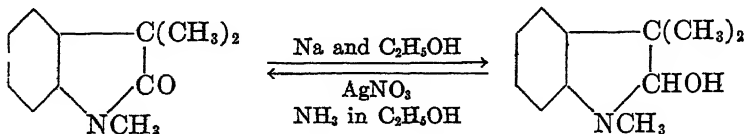


hydrolysis yields 1-methyl-3-hydroxymethyloxindole (XV).

1,3,3-Trialkyloxindoles react with the Grignard reagent to give indolinol derivatives (32, 97).

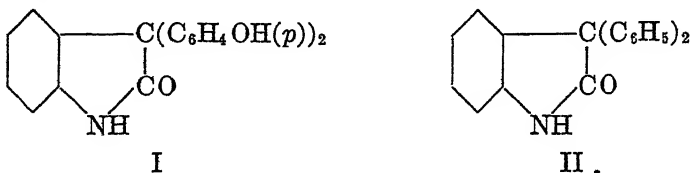


The reduction of 1,3,3-trialkyloxindoles yields indolinol derivatives in a reaction which can be reversed (28, 31, 39).



F. 3,3-Diaryl derivatives of oxindole

Baeyer and Lazarus (11, 12) and subsequently Liebermann and Danaila (135) found that isatin condenses with toluene, phenol, resorcinol, anisole, α -naphthol, and secondary and tertiary aromatic amines to give oxindole derivatives of the type of phenolisatin (3,3-bis(4'-hydroxyphenyl)oxindole) (I).



In general it was assumed, in agreement with Baeyer, that these condensation products were 3,3-derivatives of oxindole (on the other hand compare Sen (170)), but it remained for Inagaki (90, 91, 92, 93, 94, 95) to establish definitely their structure. Inagaki synthesized the parent compound of the series (II) by treating a benzene solution of 3,3-dichloro α -oxindole with aluminum chloride. The method had been employed by Inagaki and by others (154, 186, 193) for the synthesis of other members of the series. Other 3,3-diaryl derivatives of oxindole have been prepared by Candea (71), Steopoe (177), and Gabel and Zubarovski (55).

3,3-Diphenyloxindole (II) has also been prepared from 3,3-dibromo α -oxindole and benzene through the agency of aluminum chloride (193). It has also been found that the same compound (II) is formed through the action of benzene and aluminum chloride on isatin (189).

Phenolisatin and its diacetyl derivative, isacene, have found use pharmacologically as mild purgatives (17, 27, 38, 65, 171, 208). A number of halogenated derivatives of phenolisatin have also been described (135, 185). Certain sulfonated members of the series are said to be of value as mothproofing agents (62).

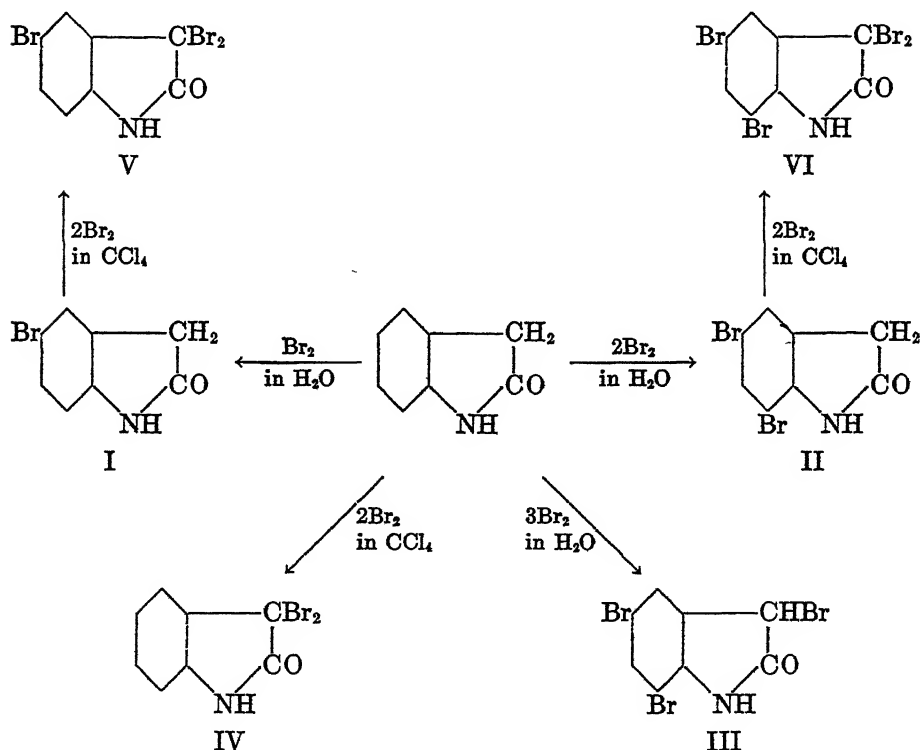
G. Halogenation, nitration, and sulfonation

In their first paper on oxindole and dioxindole Baeyer and Knop (10) described several substituted derivatives of oxindole. Since this work was done before the true structures of oxindole, dioxindole, and isatin were known, the structures of these derivatives were not determined. It also appears that in at least some cases the physical properties reported by Baeyer and Knop were seriously in error (82, 190, 193, 194), and it is doubtful that their products were obtained in a pure state.

The bromination of certain *N*-substituted oxindoles was investigated by Stollé

and coworkers (180). These investigators found that the bromination of *N*-substituted oxindoles in aqueous solution yielded derivatives with bromine substituted in position 5 when one molecular proportion of bromine was employed and in positions 5 and 7 when two molecular proportions of bromine were used. On the other hand, the bromination of *N*-substituted oxindoles in anhydrous carbon tetrachloride gave the 3,3-dibromo derivatives.

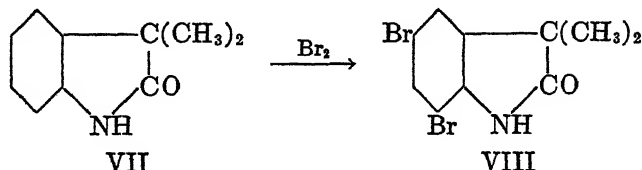
Baeyer and Knop (10) reported the preparation of a monobromoöxindole through the action of bromine water on an aqueous solution of oxindole, and reported the melting point of this derivative as 176°C. The preparation was repeated by Henze and Blair (77), who found the melting point to be 220–221°C. but did not determine the structure of the compound. The entire problem of the bromination of oxindole was recently investigated by Sumpter, Miller, and Hendrick (193), who found that this monobromoöxindole was 5-bromoöxindole (I). The use of two molecular proportions of bromine results in the preparation of 5,7-dibromoöxindole (II), while with three molecular proportions of bromine



3,5,7-tribromoöxindole (III) is obtained. When the bromination is carried out with two molecular proportions of bromine in anhydrous carbon tetrachloride, 3,3-dibromoöxindole (IV) is obtained. The bromination of I and II under similar conditions yields V and VI, respectively.

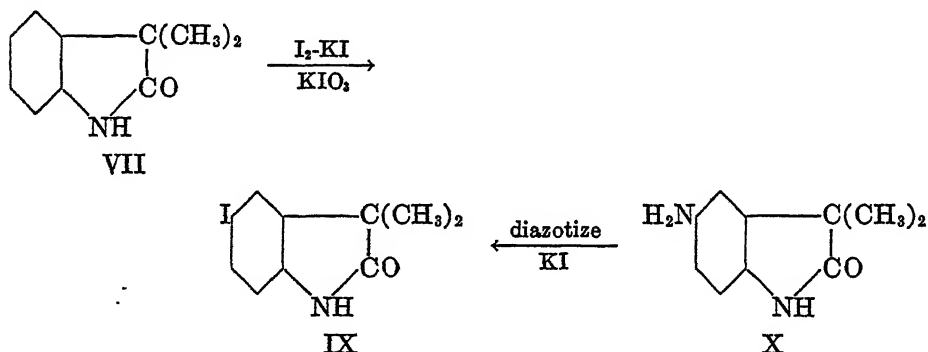
Brunner and coworkers (34) obtained a dichloro derivative of 3,3-dimethyl-

oxindole (VII) by the direct chlorination of the parent compound. They assumed that this was the 5,7-derivative, from analogy with the dibromo derivative (VIII) obtained from VII by the action of bromine. The structure of compound VIII was definitely established by its synthesis from 2,4-dibromo-

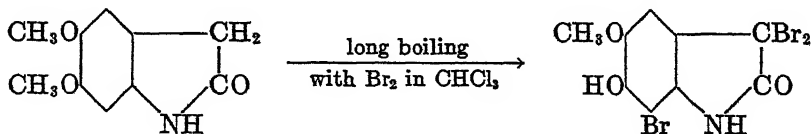


phenylhydrazine and isobutyraldehyde. Brunner also synthesized the 4,7-dibromo derivative of VII from 2,5-dibromophenylhydrazine.

The iodination of VII through the agency of iodine, potassium iodide, and potassium iodate in acetic acid gave the 5-iodo derivative (IX) (34). The structure of IX was established through its synthesis from the 5-amino derivative (X) by diazotization and replacement of the diazonium group by iodine.



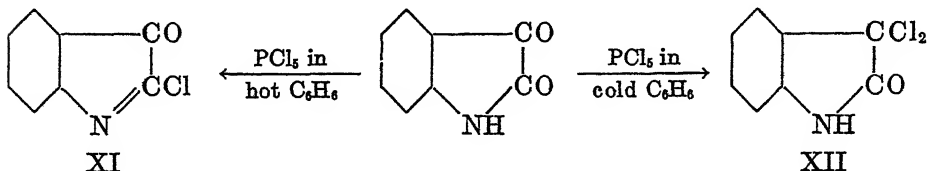
The bromination of 5,6-dimethoxyoxindole has been studied by Hahn and Tulus, who found that prolonged treatment with bromine in chloroform resulted in substitution in positions 3 and 7. The methyl group was removed from the



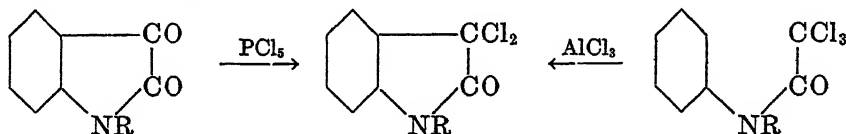
methoxyl in position 6 also.

It thus seems clear that halogen substitution in the oxindole series takes place in positions 5 and 7, respectively (34, 116, 147, 180, 190, 193, 194), and that under certain conditions the halogen can be directed to position 3. In several earlier papers Brunner (28, 30) and Schwarz (169) described bromo derivatives of 3,3-dialkyloxindoles. While the structures of these derivatives were not determined, the editors of Beilstein's *Handbuch* have assumed in several cases (16) that these compounds were the 5- and 5,7-derivatives. In the light of the evidence reviewed above this assumption seems to be justified.

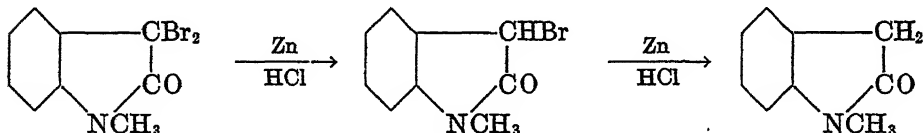
The 3,3-dichloro derivatives of *N*-alkyloxindoles were prepared by Stollé (180) through the action of calcium hypochlorite on the oxindole. The 3,3-dichloroöxindoles are also prepared quite readily from the corresponding isatin through the agency of phosphorus pentachloride. Isatin itself gives either isatin- α -chloride (XI) or 3,3-dichloroöxindole (XII), depending on the reaction conditions (6, 72).



The 1-alkyl(or aryl)-3,3-dichloroöxindoles may be prepared from the corresponding isatins or through the Stollé synthesis from the appropriate trichloroacetyl-*N*-alkylanilide.

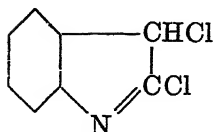


The 3,3-dibromo and 3,3-dichloro derivatives of 1-ethyloxindole, 1-methyloxindole, and 1-propyloxindole were prepared by Michaelis (152) and by Colman (40) and Fischer and Hess (52) by treating the appropriate 1-alkylindole or 1-alkylindole-2-carboxylic acid with sodium hypochlorite. Colman (40) found that reduction of 1-methyl-3,3-dibromoöxindole with zinc dust and hydrochloric acid gave 1-methyl-3-bromoöxindole and 1-methyloxindole.



The preparation of 3,3-dichloroöxindole derivatives by the combined action of chlorosulfonic acid and hydrochloric acid on isatin and isatin derivatives has been reported (66). In a subsequent patent (198) the same worker reported that the 3,3-dichloroöxindole derivatives so obtained contained at least one sulfonyl chloride grouping in the oxindole nucleus. It has been found in this laboratory (189) that the product obtained when isatin is dissolved in chlorosulfonic acid and the solution then treated with sodium chloride is not 3,3-dichloroöxindole, as reported in the first patent (66), but a compound containing sulfur. While this compound was not investigated further, this finding is in keeping with the second patent (198).

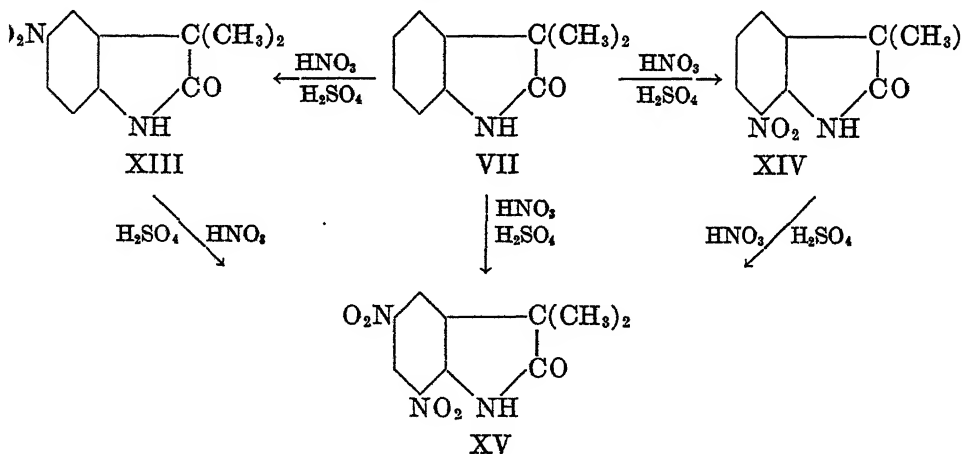
Baeyer (6) found that oxindole reacts with phosphorus pentachloride, yielding a product designated as chloroöxindole chloride. This same substance is obtained when dioxindole is treated with phosphorus pentachloride.



Chloroöxindole chloride

Oxindole was converted into a nitro derivative by Baeyer (8), who did not determine its structure. Borsche, Weussmann, and Fritzche (22) reported that the compound was 6-nitroöxindole but gave no proof other than the claim that the compound on treatment with nitrous acid gave a nitroisatin oxime supposedly different from that obtained from 5-nitroisatin and hydroxylamine. The work of Baeyer and of Borsche was repeated by Sumpter, Miller, and Magan (194), who found the work of Borsche in error. The product obtained by nitrating oxindole was definitely shown to be the 5-nitro derivative, as would be expected. 1-Methyloxindole was nitrated by Porter, Robinson, and Weyler (160). These workers were uncertain, in view of Borsche's paper (22), whether the resulting compound should be formulated as the 5-nitro or as the 6-nitro derivative. In view of the work of Sumpter, Miller, and Magan (194) on the nitration of oxindole, there seems little doubt that the product of Porter, Robinson, and Weyler was analogously 1-methyl-5-nitroöxindole.

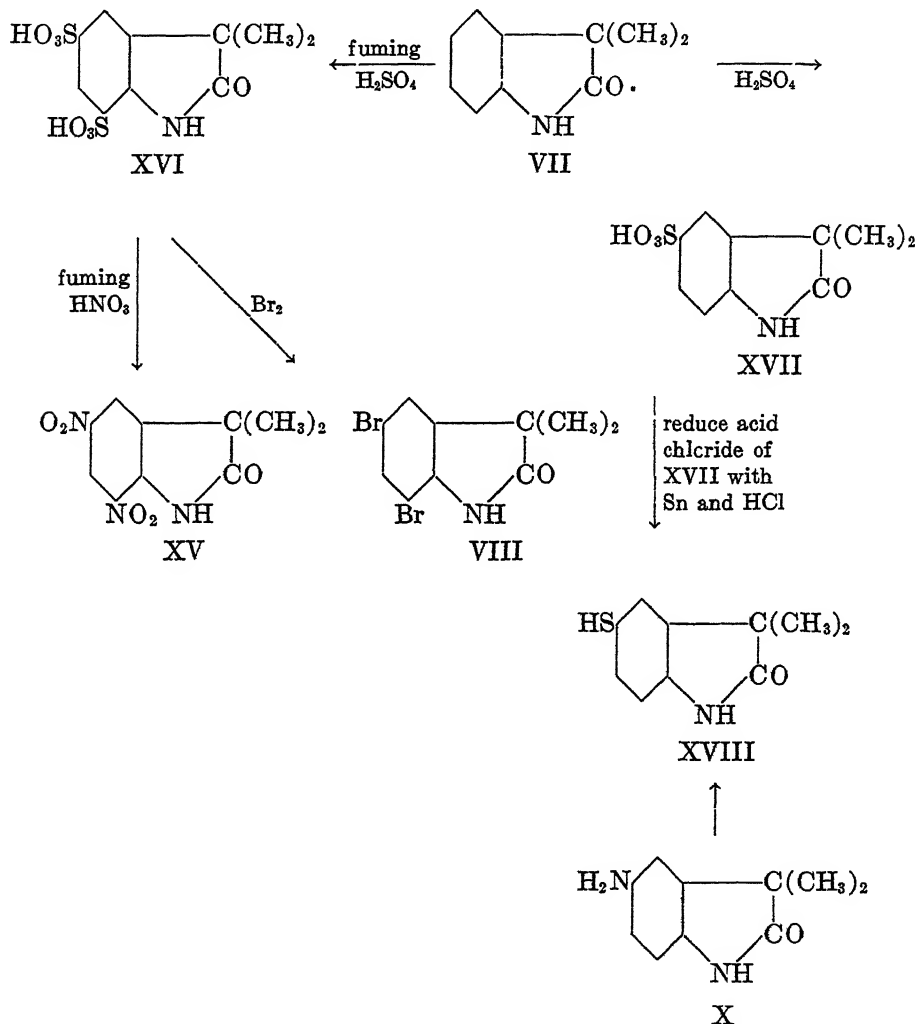
Brunner and coworkers (34) found that 3,3-dimethyloxindole (VII) with nitric acid gave two mononitro derivatives, which were identified as the 5-nitro derivative (XIII) and the 7-nitro derivative (XIV), respectively.



These two derivatives (XIII and XIV) were also synthesized from the corresponding nitrophenylhydrazines by methods which leave no doubt as to their structure. The 5,7-dinitro derivative (XV) was prepared by the nitration of VII and also by further nitration of XIII and XIV. In the light of the evidence outlined above it seems reasonable to assume that the nitration of certain other oxindole derivatives by Brunner also yielded the 5-nitro and the 5,7-dinitro

derivatives. This assumption has been made by the editors of Beilstein's *Handbuch* (16) in designating certain nitro derivatives as the 5- and 5,7-derivatives.

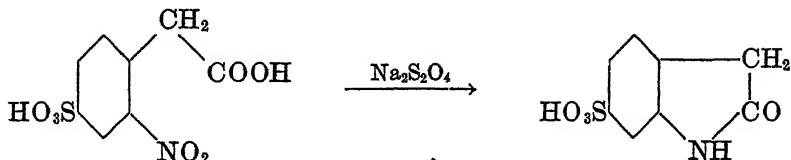
The only study of the direct sulfonation of an oxindole derivative is that made by Brunner (35). 3,3-Dimethyloxindole (VII) was sulfonated through the agency of sulfuric acid and of fuming sulfuric acid. The corresponding 5- and 5,7-disulfonic acid derivatives of VII were obtained.



The structure of the disulfonic acid (XVI) follows from its conversion into the dinitro derivative (XV) and the dibromo derivative (VIII), respectively. The structure of XVII was shown by its conversion to XVIII, which was also prepared from X through the diazonium salt.

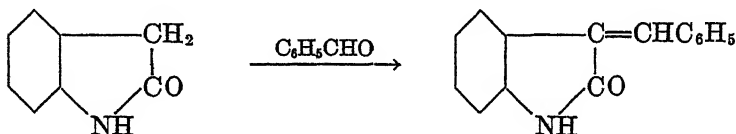
The direct sulfonation of oxindole does not seem to have been attempted.

Oxindole-6-sulfonic acid was prepared by Martinet and Dornier as indicated below (149) (compare also reference 61):



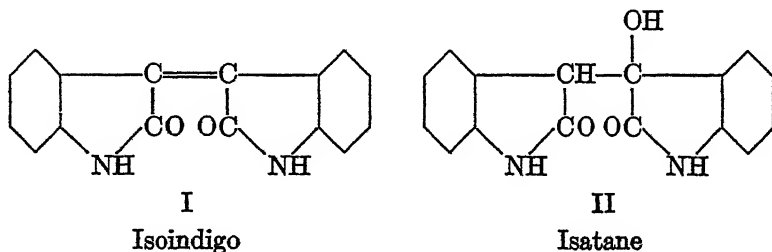
H. Condensation with aldehydes and ketones

Wahl and Bagard (200, 201, 202) found that oxindole condenses readily with benzaldehyde and with substituted benzaldehydes in the presence of piperidine to give benzaloxindoles.

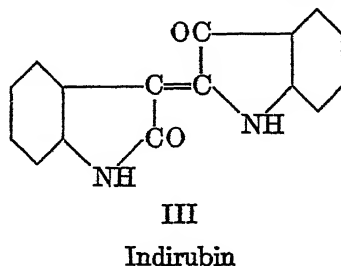


Similar condensations have been effected by Borsche (21), Wahl and Faivret (204), Wahl and Ferecean (205), Windaus and Eickel (211), Neber (155), Kliegl and Schmalenbach (112), Neber and Röcker (157), Armit and Robinson (3), Kirchner (110), Stollé (180), and Horner (89).

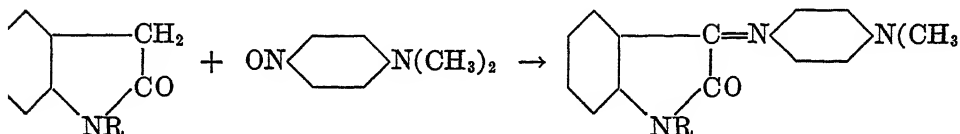
Isatin and oxindole condense in acid media (50, 71, 122, 138, 156, 200, 201, 203, 204, 205, 206) to give isoindigo (I) and in the presence of pyridine to give isatane (II) (71, 128, 132, 138, 156, 204, 206).



Oxindole and isatin chloride condense readily to give indirubin (III).



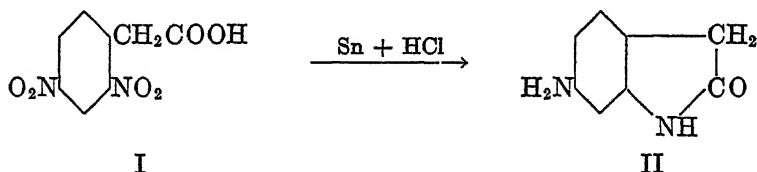
Oxindole and nuclear-substituted oxindoles condense with nitrosobenzene (156) and with *p*-nitrosodimethylaniline (156, 180) to give derivatives of isatin-3-anil.



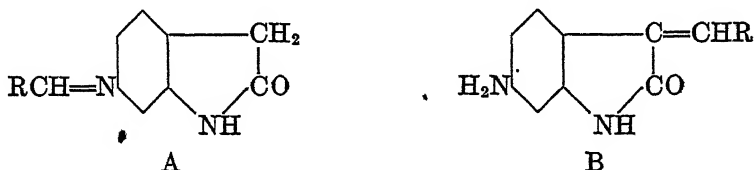
Many derivatives of oxindole have been prepared through the condensation of isatin and isatin derivatives with compounds containing active methylene groups. These reactions have already been summarized in two reviews (74, 188).

I. Amino derivatives of oxindole

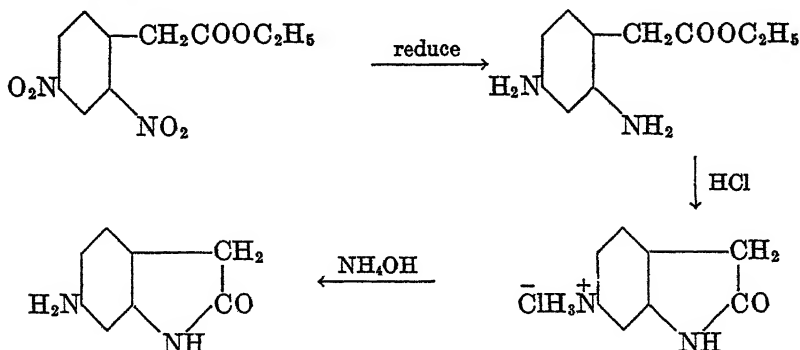
6-Aminoöxindole (II) was prepared by Gabriel and Meyer (56) by the reduction of 2,4-dinitrophenylacetic acid (I) with tin and hydrochloric acid. This method was also utilized by Parks and Aldis (158). The compound (II) was also prepared by Kishi and condensed with various aldehydes, condensation products



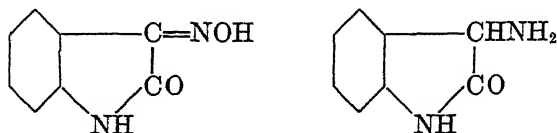
of types A and B being obtained under different experimental conditions.



6-Aminoöxindole (II) was also prepared by Ruggli and Grand (166), as shown in the following scheme:

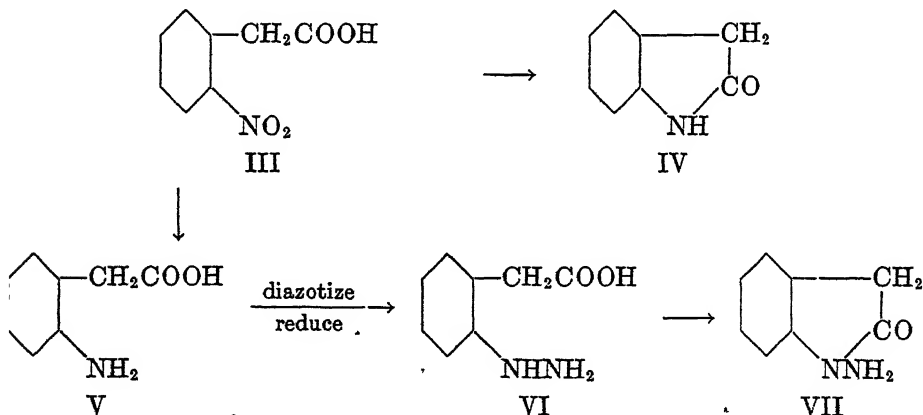


3-Aminoöxindole was first prepared by Baeyer (5, 10) by the reduction of the 3-isatin oxime obtained by the action of nitrous acid on oxindole or of hydroxylamine on isatin.

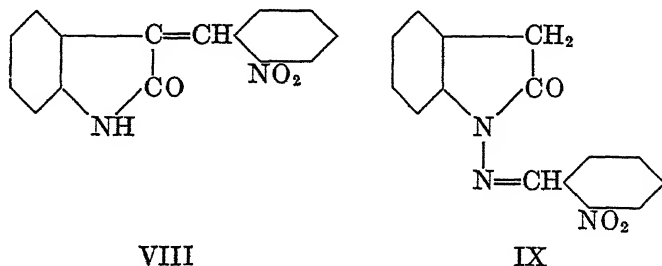


A number of 3-amino derivatives of oxindole and of substituted oxindoles have been prepared in this way by Langenbeck and his coworkers (124, 125, 126), while the method has also been used by Di Carlo and Lindwall (45).

It has been shown by P. W. Neber (155, 156) that while the reduction of *o*-nitrophenylacetic acid (III) ordinarily gives oxindole (IV) through ring closure, under proper conditions *o*-aminophenylacetic acid (V) can be obtained. When the latter compound is diazotized and reduced by stannous chloride and the product (VI) quickly distilled, 1-aminoöxindole (VII) is obtained.

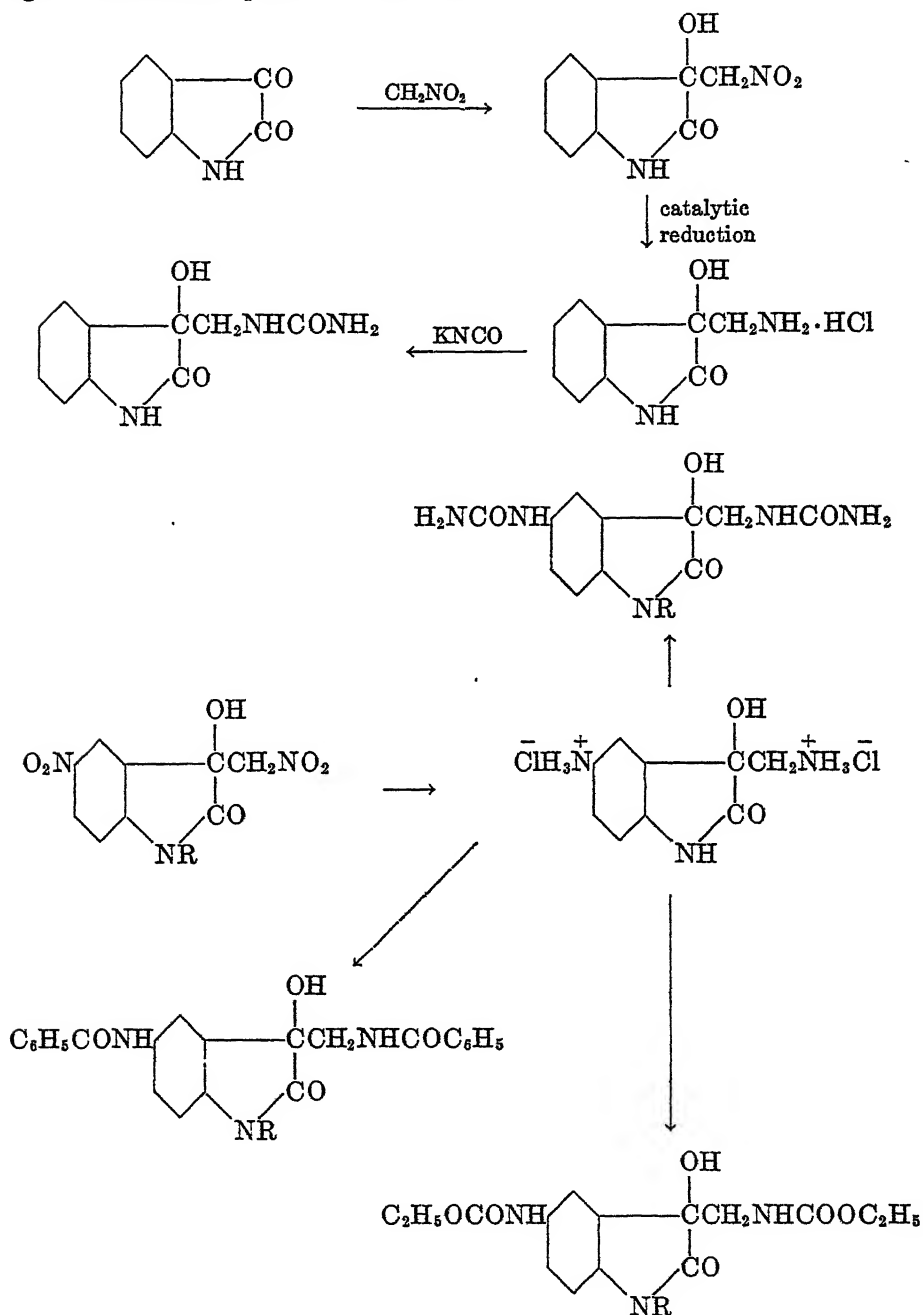


Compound V condensed with *o*-nitrobenzaldehyde to give what Neber thought was a quinoline derivative, but the product was shown by Kliegl and Schmalenback (112) to be 3-(*o*-nitrobenzal)oxindole (VIII) (m.p. 226–227°C.). On the other hand, VI and *o*-nitrobenzaldehyde condense to give IX (m.p. 170°C.).



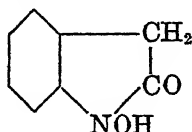
A number of derivatives of 1-aminoöxindole were prepared by Neber and Keppler (156).

Other amino derivatives of oxindole were prepared by Lindwall and coworkers (41, 42) by condensing members of the isatin series with nitromethane and reducing the condensation product to the amine.



J. Hydroxy derivatives of oxindole

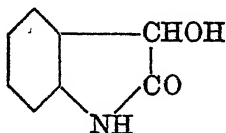
1-Hydroxyoxindole was obtained by Reissert (163, 164) by the reduction of



1-Hydroxyoxindole

o-nitrophenylacetic acid with zinc and hydrochloric acid. Oxindole was also obtained in the same reduction. Di Carlo (44) found that catalytic reduction of *o*-nitrophenylacetic acid under certain conditions gave 1-hydroxyoxindole, along with oxindole. 1-Hydroxyoxindole was converted to 1-acetoxyoindole through the action of acetic anhydride and to 1-methoxyoxindole through the agency of methyl sulfate (76, 164). 1-Acetoxyoindole was reduced to oxindole by the action of zinc dust and acetic acid (78).

3-Hydroxyoxindole (dioxindole) was first prepared by Baeyer and Knop (10) through the reduction of isatin by the action of sodium amalgam in alkaline medium. Reduction of isatide by sodium amalgam also yielded dioxindole (10). Isatin has also been reduced to dioxindole, by Heller (75), using zinc and acetic



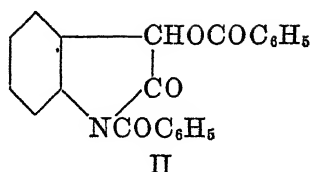
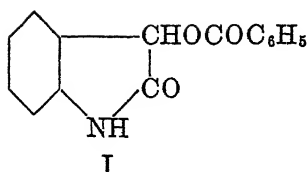
Dioxindole

acid, and by Marschalk (144, 142) and Kalb (102), using sodium hydrosulfite as the reducing agent.

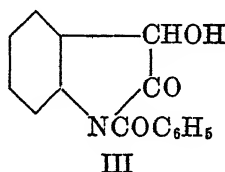
Isatin-4-carboxylic acid is reduced by sodium amalgam to dioxindole-4-carboxylic acid. The latter compound disproportionates in boiling alcoholic solution, yielding isatin-4-carboxylic acid and oxindole-4-carboxylic acid (26).

The reduction of 1-methylisatin and 1-ethylisatin by zinc and hydrochloric acid gives the corresponding 1-alkyldioxindole (40, 152, 189). Sodium hydrosulfite can also be used for the reduction of various isatin derivatives to the corresponding dioxindoles (85, 189, 190, 204). The isolation of the *d*- and *l*-enantiomorphs of dioxindole, which is obtained as the racemic dioxindole in all of the above preparations, has been effected by McKenzie and Stewart (140). Measurement of the oxidation potential of dioxindole has been made by Fieser (51).

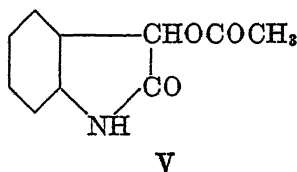
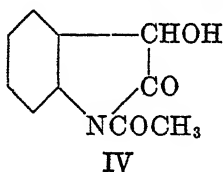
Dioxindole reacts with benzoyl chloride to give a benzoyl derivative (m.p. 134°C.), which has been shown by Heller (75, 81) and by McKenzie and Stewart (140) to be 3-benzoyldioxindole (I). 1,3-Dibenzoyldioxindole (II) (m.p. 170°C.) was prepared by Heller (75) and by McKenzie and Stewart through the Schotten-Baumann reaction.



An isomer of I (m.p. 104°C.) was prepared by Hill and Sumpter (85) through the action of benzoyl chloride on the sodium salt of either dioxindole or isatide. The molecular weight of the substance corresponds to that of a benzoyl derivative of dioxindole and not to that of an isatide derivative (189). It is possible that this compound is 1-benzoyldioxindole (III), although definite proof of structure is lacking as yet.

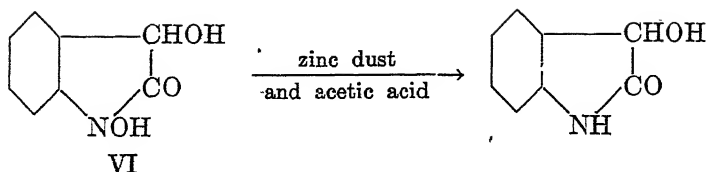


3-Acetyldioxindole (V) (m.p. 127°C.) was prepared by Suida (183, 184) through the action of acetic anhydride on dioxindole. The compound was described by Suida as being the 1-acetyl derivative (IV). Heller later reported that Suida's



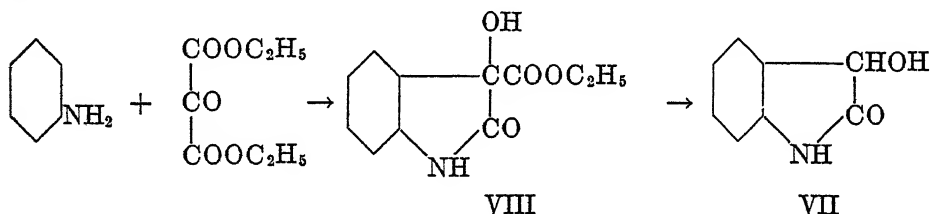
acetyldioxindole was the 3-acetyl derivative (V) (references 74 (page 18) and 81). 1-Acetyldioxindole (IV) (m.p. 127°C.) was prepared by Sumpter (189) through the reduction of 1-acetyl isatin with sodium hydrosulfite. While the melting points of IV and V are identical, the preparations are not identical, as is shown by the fact that a mixture of the two exhibits a marked depression in melting point. Since the method of preparation of IV leaves little doubt of its structure, it follows that Heller (74, 81) and McKenzie and Stewart (140) were correct in their conclusion that Suida's acetyldioxindole is V and not IV. An acetyldioxindole (m.p. 127°C.) was obtained by Bamberger and Lindberg (14) and described by these workers as "possibly 1-acetyldioxindole". From the method of preparation and physical properties this substance might have been either IV or V. This point can only be settled by the repetition of the work of Bamberger and Lindberg. The acetyl derivatives of a number of 1-alkyldioxindoles have been described by Stollé and Merkle (181).

1-Hydroxydioxindole was prepared by Heller (77) through the reduction of *o*-nitromandelic acid by zinc dust and ammonium hydroxide. This compound (VI) is reduced to dioxindole by the action of zinc dust and acetic acid.



Through the reduction of their "6-nitroisatin" by hydrogen in the presence of nickel catalyst, Rupe and Apotheker (167) obtained a compound which they regarded as being 6-aminodioxindole. Since Sumpter and Jones (191) demonstrated that Rupe's 6-nitroisatin was in reality 5-nitroisatin, it follows that the hydrogenation product was probably 5-aminodioxindole. The latter compound was also prepared by Hartmann and Pannizzon (73).

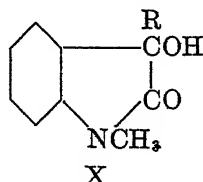
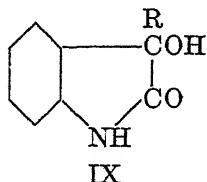
Another method for the synthesis of dioxindole and its derivatives is provided by a procedure developed by Martinet and coworkers (20, 67, 144, 145, 147, 149). Aniline or a substituted aromatic amine is condensed with the ethyl or methyl ester of oxomalonate. On treatment with alkali the resulting compound (VIII) gives dioxindole (VII) in the absence of oxygen. In the presence of oxygen isatin results through oxidation of VII. The reaction has also been studied



by Kalb (102, 104), by Halberkann (70), by Hinsberg (86), and by Langenbeck (122, 123). Heller (80a) found that VIII could also be prepared by the hydrolysis and esterification of the product obtained by the condensation of isatin with hydrocyanic acid.

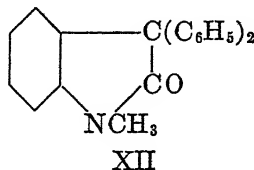
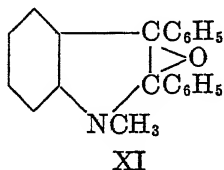
Dioxindole is converted into isatin β -phenylhydrazone by heating with phenylhydrazine (75, 146).

3-Alkyl-3-hydroxyoxindoles (IX) are readily prepared by the action of Grignard reagents on isatin (85, 115, 116, 119, 120, 121, 154, 174, 175, 176, 182, 186, 187). (For other syntheses of 3-phenyl-3-hydroxyoxindole see references 13 and



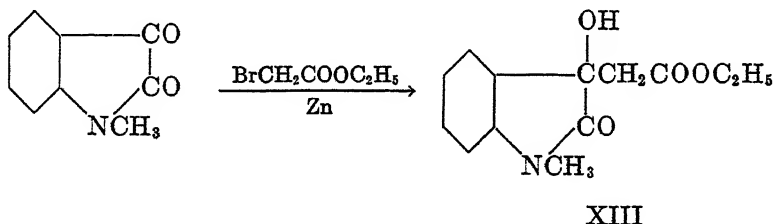
103.) Kohn (120) found that when phenylmagnesium bromide and *N*-methylisatin were allowed to react in equimolecular proportions, the analogous 1-methyl-3-phenyl-3-hydroxyoxindole (X) was obtained. On the other hand, when *N*-methylisatin was treated with excess Grignard reagent Kohn found that both

carbonyl groups in the isatin molecule reacted, yielding XI. It was subsequently found by Myers and Lindwall (154) that Kohn's product was in reality a mixture of XI and the rearrangement product XII. The latter substance was also obtained by Reeves and Lindwall (162a) by the action of phenylmagnesium bro-

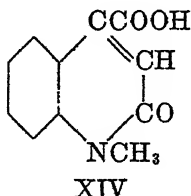


mide on *N*-methyl benzoylformanilide. The reactions of *N*-substituted isatins with Grignard reagents have also been studied by Inagaki (96), by Stollé (182), and by Sumpter (186, 187).

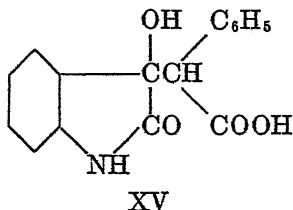
The Reformatsky reaction was employed by Myers and Lindwall (153) to obtain the ethyl ester of 3-hydroxy-1-methyloxindolyl-3-acetic acid (XIII). Hydrolysis of this ester brings about ring opening and subsequent closure to give



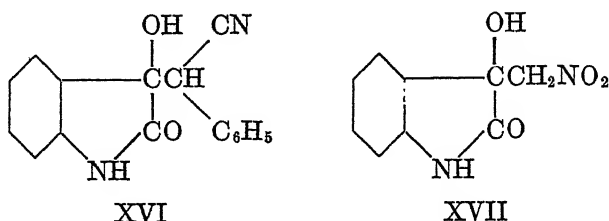
the quinoline derivative (XIV).



Isatin undergoes an aldol type of reaction with many compounds containing active methylene groups, yielding 3-hydroxy derivatives of oxindole (reference 188, page 413). For example, Zrike and Lindwall (213) found that isatin condenses with ethyl phenylacetate to give the product XV.



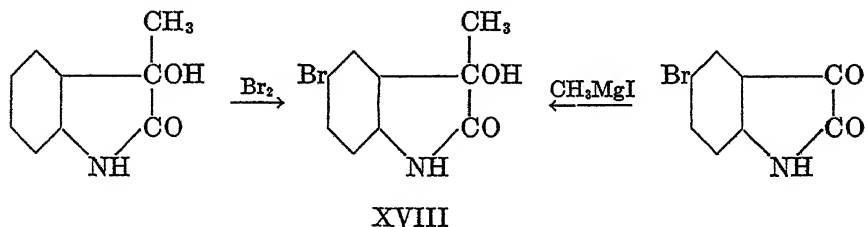
Hill and Samachson (84) found that phenylacetonitrile condensed with isatin to give compound XVI.



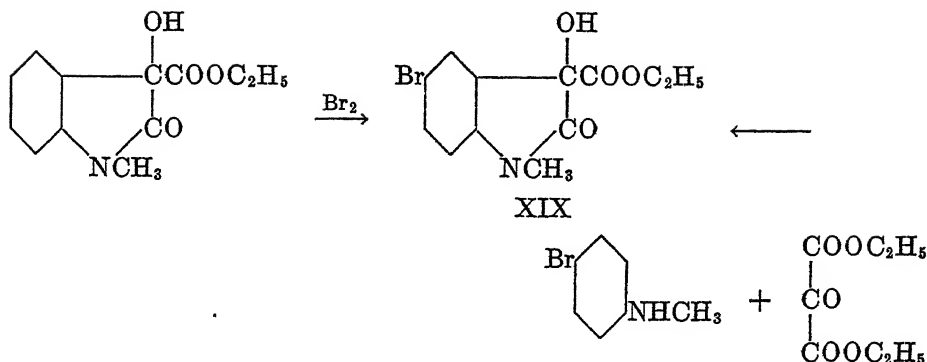
Similar aldol-like condensation products (XVII) were obtained by Lindwall and coworkers when isatin and substituted isatins were condensed with nitromethane and nitroethane. Other 3-hydroxyoxindole derivatives were prepared by Lindwall and coworkers (25, 47, 48, 136, 213), by Baumen (15), and by Schöenberg, Schütz, Arend, and Peter (168).

Baeyer and Knop (10) described the preparation of a number of substituted dioxindoles by direct substitution. Most of these preparations have not been repeated. The preparation of the monobromodioxindole of Baeyer and Knop (10) has been repeated in this laboratory (190) and the melting point reported by Baeyer found to be far too low. That the product of this bromination is 5-bromodioxindole has been shown by the preparation of the same compound by the reduction of 5-bromoisatin with sodium hydrosulfite. Further proof of structure was provided by the fact that the bromodioxindole yielded 5-bromoisatin phenylhydrazone when heated with phenylhydrazine. It was further found that the dibromo derivative (from dioxindole and two molecular proportions of bromine) was the 5,7-dibromo derivative. This was established by its preparation from 5,7-dibromoisatin through reduction as well as its conversion to 5,7-dibromoisatin phenylhydrazone through the agency of phenylhydrazine.

Kohn (116) found that the 3-alkyl-3-hydroxyoxindoles brominate in position 5. 5-Bromo-3-methyl-3-hydroxyoxindole (XVIII) was prepared equally well by the action of bromine on 3-methyl-3-hydroxyoxindole and from the Grignard complex resulting from the action of methylmagnesium iodide on 5-bromoisatin.



Martinet (147) found that ethyl 5-bromo-3-hydroxy-1-methyloxindole-3-carboxylate (XIX) could be prepared by the bromination of the parent compound or by the condensation of ethyl oxomalonate with *N*-methyl-*p*-bromoaniline.



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SYNTHETIC ESTROGENS AND THE RELATION BETWEEN THEIR STRUCTURE AND THEIR ACTIVITY

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CONTENTS

I. Introduction.	481
II. Historical.....	484
III. Hexestrol.....	486
A. Discovery.	486
B. Syntheses (methods other than hydrogenation).....	490
C. Optical isomerism.	493
IV. Stilbestrol	495
A. Syntheses.....	495
1. Syntheses from anisoin.	496
2. Introduction of anisyl group by Friedel-Crafts reaction.....	497
3. Introduction of anisyl group by Grignard reaction.. . . .	498
4. Syntheses involving retro-pinacolin rearrangements....	498
5. Syntheses involving miscellaneous rearrangements	502
B. <i>Cis-trans</i> isomerism of stilbestrol	504
C. Analytical methods for stilbestrol determination	509
D. Hydrogenation of stilbestrol	509
1. Hydrogenation of aliphatic double bond.	509
2. Hydrogenation of aromatic rings.	512
V. Variation of fundamental structure.. . . .	513
A. Variation of ring substituents	513
1. Acylation and alkylation of phenolic hydroxyl groups.. . . .	513
2. Position isomers and homologs with additional ring substituents....	519
B. Variation of aliphatic chain.	533
1. Homologs of stilbestrol and hexestrol.....	533
2. Introduction of functional groups.	537
3. Location and number of aliphatic double bonds.....	539
VI. Triphenylethylene derivatives.....	542
VII. Diphenylmethane derivatives.	551
VIII. Diphenylpropane derivatives	558
IX. Ring-closed analogs.....	565
X. Relation of synthetic estrogens to carcinogenic substances.....	572
XI. Metabolic conversions and efficacy by various routes.....	574
XII. Appendix... ..	580
XIII. References.....	583

I. INTRODUCTION

In 1933 Cook, Dodds, and Hewett (35) stated that "it seems likely that a whole group of substances of related chemical constitution will be found to have estrus-exciting properties and the synthetic production of such substances would probably be of considerable clinical value." This prediction proved to be fully correct. During the eleven years following this first publication on synthetic

estrogens the field has continually grown in importance as well as in scope, and today there are several hundred substances known to possess estrogenic activity. It is common knowledge that several among these have acquired considerable theoretical and practical importance. The discovery of the estrogenic activity of stilbestrol in 1938 by Dodds, Goldberg, Lawson, and Robinson (58) constitutes by far the most important single advance of this research. Further studies by the same group of investigators as well as by others soon led to the synthesis of a few closely related compounds of similar potency. Since that time research on synthetic estrogens has clearly shown a trend of elaborating and improving over these known structures rather than venturing into entirely new fields. There are a few exceptions to this statement, but even such structures which, strictly speaking, are neither stilbene nor dihydrostilbene derivatives, are nevertheless closely related. Among these are derivatives of diphenylmethane, diphenylpropane, triphenylethylene, and certain ring-closed analogs. Such compounds have also been included in this review because the correlation of chemical structure and biological activity of the stilbenes could not be fully discussed without including them and, conversely, their significance lies in their relationship to the estrogens of the stilbene and dihydrostilbene types. Not included in this review are structures more closely related to the natural hormones than to stilbestrol, as well as other miscellaneous derivatives of benzene, diphenyl, diphenyl ether, acenaphthene, fluorene, phenanthrene, and anthracene. These types of compounds have been treated comprehensively by Masson (118a).

The great amount of painstaking detailed research done in this field lies in the importance of synthetic estrogens as therapeutic agents, an importance which is mainly due to their greater availability and to their efficacy on oral administration. For a number of years a spirited and intense discussion has been under way between those who advocate continued and exclusive use of natural estrogens of the steroid type and those who sponsor their replacement by synthetics of non-steroid structure; however, this discussion concerns therapeutic merits and is beyond the scope of this review, which is concerned with correlating the data on chemical structure and estrogenic activity.

The endeavor to review this field at the present time is prompted mainly by three reasons: firstly, the literature on estrogenic stilbenes and related compounds has become so extensive as to make it desirable to review the chemical facts reported to date; secondly, the research undertaken in the field of synthetic estrogens constitutes one of the most extensive efforts to answer the cardinal question of the correlation between chemical structure and biological activity; and, thirdly, the present state of knowledge in this field appears especially opportune for drawing an intermediary balance.

Necessarily, the discussion of physiological aspects of synthetic estrogens must be limited to those bearing on the correlation of structure and potency. The physiology of synthetic estrogens in its entirety has recently been reviewed by Masson (118a). An earlier review by Wessely (219) covers the literature up to 1940. Partial aspects have since been reviewed in greater detail by Atkinson (4), Morell and Hart (133, 134), and The Council on Pharmacy and Medicine of the American Medical Association (38).

As mentioned earlier, the efficacy on oral administration is one of the main assets of synthetic estrogens. Consequently, assays of oral activity are an important part of the evaluation of any new compound. In order to limit the discussion of physiological aspects and because information on parenteral efficacy is more generally available, comparative oral activities will be omitted from the chemical discussion of the various estrogens; they will be briefly treated in a separate chapter, together with some metabolic conversions intimately related to the oral efficacy.

The question arises if it is feasible to correlate the wealth of information on estrogenic activities. Generally speaking, the physiological results of various authors employing various methods may be compared only with a fairly wide margin of error. This is particularly true for estrogenic activities, where many variations arise from differences in technique as well as in interpretation as practiced in different laboratories. The assay procedure most frequently adopted is based on the method of Allan, Dickens, and Dodds (1a). It consists in the subcutaneous administration of a solution of the test sample in oil, under standardized conditions, to ovariectomized female rats. The rat unit is defined as the minimum amount of an estrogenic substance required to produce full estrous response in 100 per cent of the animals tested. Campbell (21) specified injection in six doses in sesame oil solution during 3 days. A number of investigators modified this standard procedure, used mice or other test animals instead of rats, or based their reported activities on a positive response in less than 100 per cent. The state of "full estrous response" alone, i.e., the degree of cornification of the vaginal smear, requires strict definition. Furthermore, questions of solubility and rates of absorption as well as of excretion need to be considered.

For the purpose of roughly comparing the relative activities of compounds prepared in their own laboratory, Dodds *et al.* (59) reported the activities in approximate rat units per gram of estrogenic substance. This practice has the advantage of expressing the highest degree of potency by the highest numerical value. It has occasionally been adopted by others, though the majority of authors preferred to report the potency in terms of minimal doses in micrograms required for a certain response in a given animal, when the smallest numerical value signifies highest potency. By dividing 1,000,000 by this latter expression it may be converted into the former. One might be tempted thus to evaluate all data reported in the literature by a common expression, but in the opinion of Dodds (147) and other workers in the field it would not be sound to do this with results from different laboratories, and this is quite evident from closer examination of individual data. The quantitative data expressed in various units are reproduced here as reported in the literature and may permit a quantitative comparison within series of compounds reported from *one* laboratory. A comparison of quantitative figures of *various* origins will be useful only for obtaining a rough indication.

Because the unit is expressed in dosage weight irrespective of body weight, naturally the same dose will have the greater effect on the smaller animal. Consequently the rat unit is larger than the mouse unit, but the exact ratio of the two units varies for different estrogens. The relative activity of various estro-

gens in humans is of prime importance, as most of the research was undertaken with the objective of developing new estrogens for clinical use. In view of the marked difference in relative response by species as closely related as rats and mice, it is obvious that animal experiments serve only for the purpose of screening compounds for therapeutic trial. The clinical results will not be discussed here, but some of the problems involved may be illustrated by the fact that one of the more recently developed estrogens was assayed clinically by the almost exclusive use of the "subjective" method, evaluating the subjective reports of patients, when treated at one dose level, regarding the manifestation or lack of certain desirable as well as undesirable symptoms. It was found difficult to correlate symptomatic relief with objective results as found in vaginal smears.

The sequence of topics in discussing the various compounds has been chosen for didactic reasons. The historical development and stimulation of research by the discovery and identification of hexestrol among the demethylation products of anethole made it desirable to treat hexestrol before stilbestrol; nevertheless, the latter may be considered the central topic. While the more important substances are discussed in detail, an attempt has been made to summarize in tabular form all compounds relevant to the subject. The methyl ethers of the phenolic hydroxyl derivatives have been omitted from the tables wherever they were obtained as intermediates only, without being of special interest as estrogens.

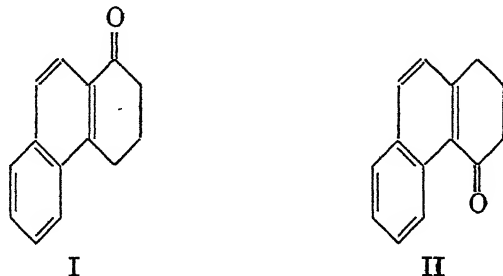
A few words will be in order regarding adoption of the abbreviated name "stilbestrol" instead of "diethylstilbestrol" as originally proposed by Dodds, Robinson, and coworkers (58). These authors gave the name "stilbestrol" to the "mother-substance" of the series, 4,4'-dihydroxystilbene, and consequently called the diethyl derivative "diethylstilbestrol." Actually, the basic name "stilbestrol" has been used only rarely in conjunction with homologs of 4,4'-dihydroxy- α,β -diethylstilbene. The Council for Pharmacy and Medicine of the American Medical Association (38) designated "diethylstilbestrol" as the officially accepted name. On the other hand, for the same compound, the abbreviated name "stilbestrol" has been increasingly used in the medical as well as in the chemical literature, including its use by Dodds *et al.* (26, 52, 56). Therefore, and for the sake of briefness, this practice has been extended to this review.

II. HISTORICAL

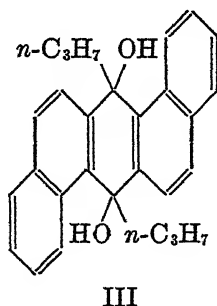
Even before the structure of the natural female sex hormones had been fully elucidated, Cook and Dodds with their collaborators (32, 34, 35) undertook to synthesize more readily accessible estrogenic substances of simpler structure. This endeavor was encouraged by the fact that estrogenic activity, being shared by a group of sterols, was apparently less specific than other hormonal activity. It was therefore not unreasonable to expect this lack of specificity even to extend to other classes of compounds. However, quoting the British authors, "... it would have been difficult to imagine that so complicated a series of effects involving an orderly sequence such as that of the estrous phenomena can be induced by anything other than the appropriate hormone."

This work was undertaken for the additional reason of studying the correlation between estrogenic and carcinogenic substances. The structural side of this relationship will be discussed later. Cook, Dodds, and Hewett (32, 35) first examined compounds containing the same ring system as carcinogenic hydrocarbons but with one or more rings hydrogenated and with polar groups present.

The first compounds of non-steroid structure reported in 1933 by these authors to possess estrogenic activity were 1-keto-1,2,3,4-tetrahydrophenanthrene (I) and the corresponding 4-keto derivative (II).



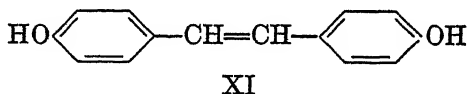
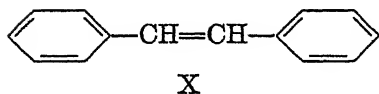
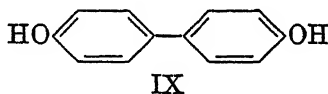
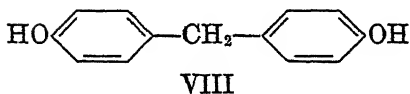
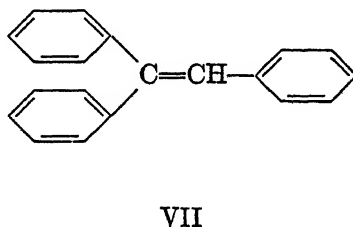
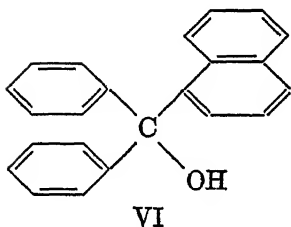
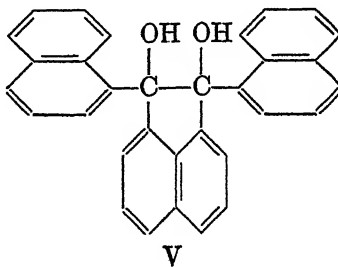
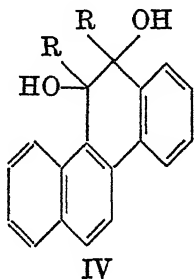
Their activity is low compared with that of estrone, and it was later recognized that activities of such low order are common to a very large number of compounds. The same authors found considerably higher activity for a series of 9,10-dihydroxy-9,10-dialkyl-9,10-dihydrobenzanthracenes. Cook, Dodds, Hewett, and Lawson (34) studied the effect of various alkyl groups on estrogenic activity; in this series the optimal activity is reached with the di-*n*-propyl derivative (III).



Extending the biological assays to other species, Cook, Dodds, and Greenwood (33) contributed to the recognition of the fundamental fact that there exist practically no qualitative differences between natural and synthetic estrogens. Among the great number of physiological reactions due to natural estrogens only isolated instances (130) have been reported of a qualitative difference between the two types of estrogens.

Between 1933 and 1938 Dodds and his collaborators (36, 50, 55, 61, 62, 64), as well as others, synthesized a large number of compounds, many of which were found to possess weak activity. Among these, only the following compounds

may be mentioned as being representative of the development away from structures still containing the phenanthrene skeleton and towards the simpler, yet infinitely more potent, stilbene derivatives: 11,12-dihydroxy-11,12-dialkyl-11,12-dihydrochrysene (IV), 1,3-dihydroxy-1,2-di- α -naphthylacenaphthene (V), diphenyl- α -naphthylcarbinol (VI), triphenylethylene (VII), bis(4-hydroxyphenyl)methane (VIII), 4,4'-dihydroxydiphenyl (IX), stilbene (X), 4,4'-dihydroxystilbene (XI).

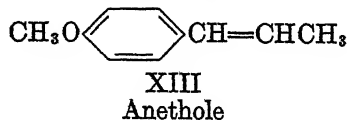
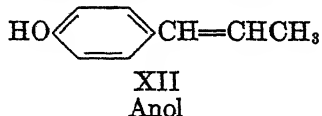


At this stage of the work, it became apparent that simple phenols and phenolic stilbene derivatives were worthy of special attention.

III. HEXESTROL

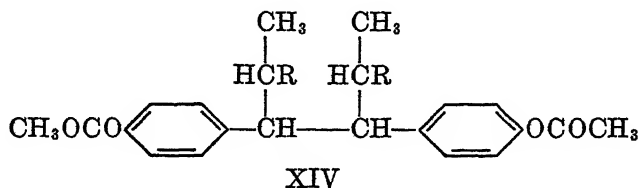
A. DISCOVERY

In 1937 Dodds and Lawson (62, 64) observed that different preparations of *p*-propenylphenol (anol) (XII) gave contradictory biological results. Their prepara-

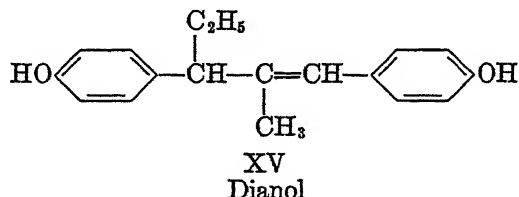


tion had been obtained from the biologically inactive anethole (XIII) by demethylation with potassium hydroxide and alcohol. Shortly thereafter, Serini

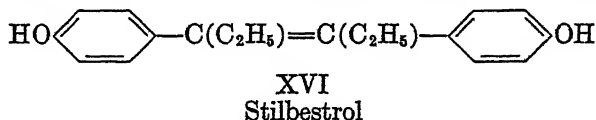
and Steinruck (177, 187) described the demethylation of anethole by means of Grignard reagents and, after acetylation of the resulting phenols, the production of crystalline products of considerable potency. With ethylmagnesium iodide, the product $C_{26}H_{34}O_4$, m.p. $186^\circ C$., was obtained; with propylmagnesium iodide, the product $C_{28}H_{38}O_4$, m.p. $175^\circ C$. It was postulated that the two products were formed by dimerization of two molecules of anol with simultaneous addition of two ethyl (propyl) groups, resulting in compounds most likely of the structure shown in formula XIV.



These products were active in doses of 5-10 micrograms (in rats), and were not identical with those obtained by Dodds and Lawson (62), who had observed substantially higher activities, though one product may have been the diacetate of the demethylation product of the latter workers. Dodds and Lawson (63), after being informed of an observation by Serini and Steinruck, confirmed that the mother liquors of their crystallized product contained the extremely potent fractions, while pure anol is only weakly active. This result was corroborated by Zondek and Bergmann (233), while Supniewski and Hano (209) apparently assayed crude preparations and found high activity. Campbell, Dodds, and Lawson (23) showed that the high potency of their crude compound could not be due to the presence of dianol (XV), which showed activity only in 100-microgram doses.



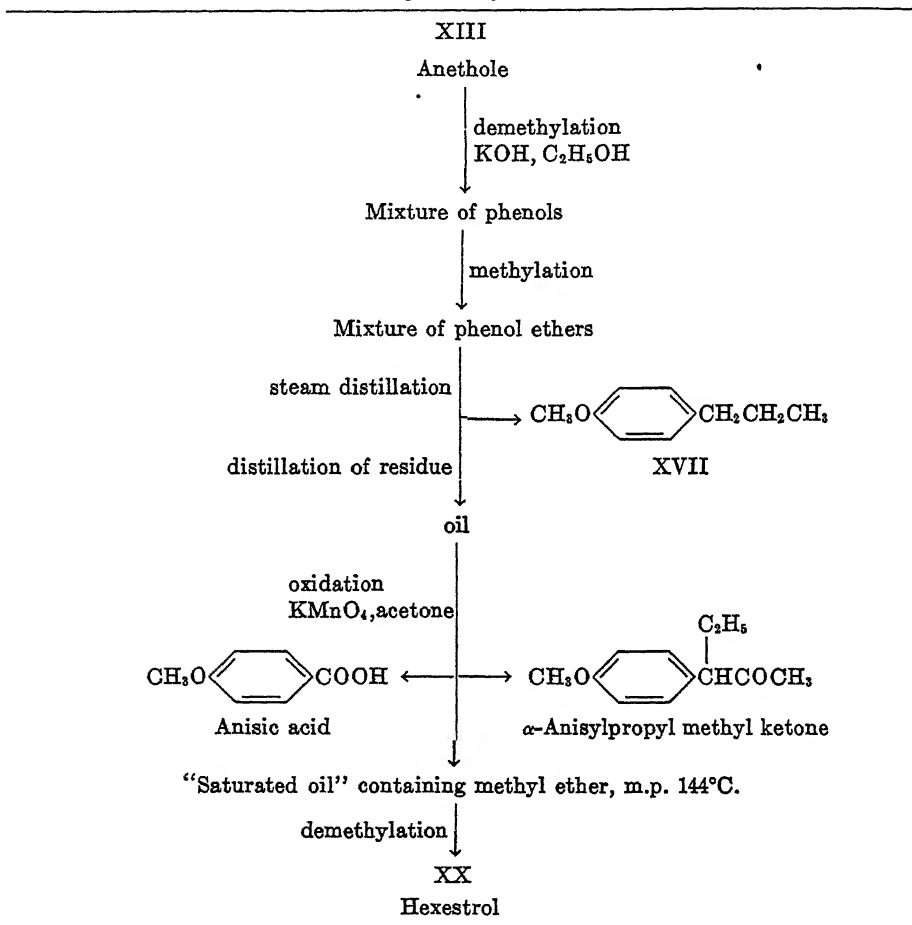
Shortly thereafter, in 1938, Dodds, Goldberg, Lawson, and Robinson (58) in a preliminary report published their synthesis of stilbestrol (4,4'-dihydroxy- α,β -diethylstilbene) (XVI). This investigation marked the opening of a new



field of research with the objective of elaborating on the stilbestrol structure and possibly attaining even higher activities. Yet it still remained to identify the nature of the compound responsible for the activity of impure anol prepara-

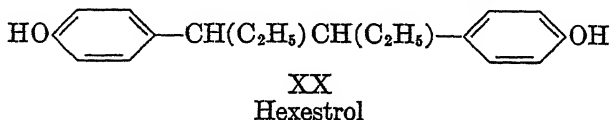
tions. Campbell, Dodds, and Lawson (24) solved this problem by careful fractionation of the demethylation products of anethole according to the scheme shown in table 1.

TABLE 1
Demethylation of anethole

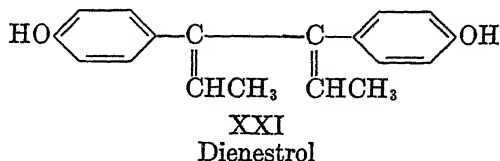


The phenol (XX), m.p. 184–185°C., proved fully active in doses of 0.2 microgram and thus accounted for the relatively high activities occasionally observed with impure preparations of anol. The compound was called hexestrol and remains to this day one of the most potent estrogens known.

The structure of hexestrol as 3,4-di(*p*-hydroxyphenyl)hexane (XX) was established by the proof of its identity with the hydrogenation products of stil-

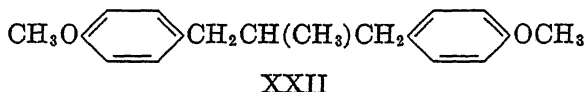


bestrol and of 3,4-di(*p*-hydroxyphenyl)-2,4-hexadiene (XXI), synthesized by Dodds *et al.* (59, 60) and to be discussed later. The occurrence of hexestrol



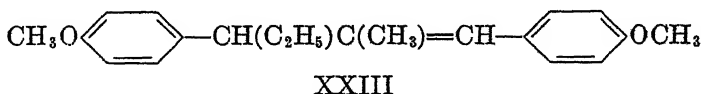
among the demethylation products of anethole was studied in greater detail by Campbell, Dodds, and Lawson (25). In addition to hexestrol (m.p. 184–185°C.) another saturated phenol was found with m.p. 122°C. (also 128°C.), later identified as the optical isomer of hexestrol and found to be considerably less active.

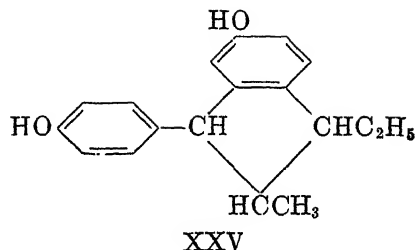
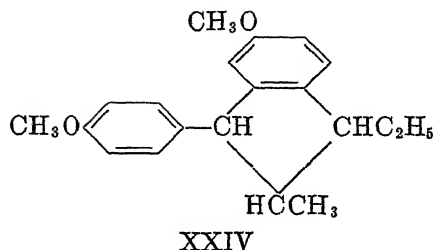
Two hydrogen atoms are required for the formation of hexestrol during the demethylation of anethole as well as for the simultaneous formation of 1-(*p*-anisyl)propane (XVII), as isolated by Campbell *et al.* (24); the origin of these hydrogen atoms is not yet fully understood, disproportionation being one possible explanation. Another saturated dimerization product formed by prolonged heating of anethole has been more recently obtained by Campbell (20) and identified as 1,3-di(*p*-anisyl)-2-methylpropane (XXII); it is only feebly active.



The isolation and structure elucidation of hexestrol are a remarkable feat in view of the fact that this product of linkage between two α -carbon atoms of anethole occurs among the demethylation products in 0.01 to 0.02 per cent yield only.

In this connection it may be of interest to note two other dimerization products of anethole (XIII) which have been the subject of recent studies. One of them is an oil, called isoanethole, and its structure (XXIII) was established by Goodall and Haworth (87); thus, isoanethole is the dimethyl ether of dianol (XV) mentioned earlier, and is formed by the linkage of one α - and one β -carbon atom. The other dimerization product of anethole is crystalline and is called metanethole; it is, according to Baker and Enderby (7) and confirmed by Mueller and Richl (137), the phenylindane derivative (XXIV) formed by linkage involving α - as well as β -carbon atoms. No assays of estrogenic activity of the demethylated metanethole ("metanethol") (XXV) have been reported, but the compound is of interest in connection with other active phenylindane derivatives to be discussed later. According to Polak (201) metanethole exists in a sublimable and a non-sublimable form; this may indicate the presence of still another isomer.



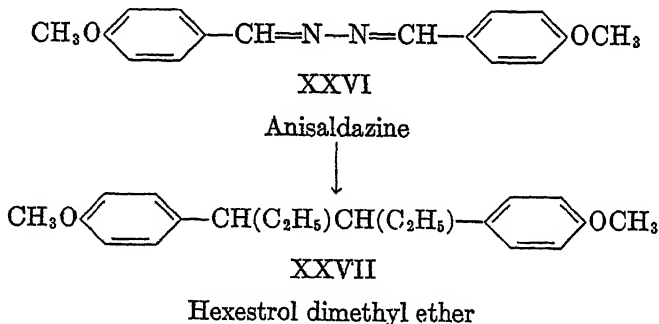


Sempronj *et al.* (186) described a product of unknown structure formed by spontaneous polymerization of anol (XII) and active in doses between 14 and 20 micrograms.

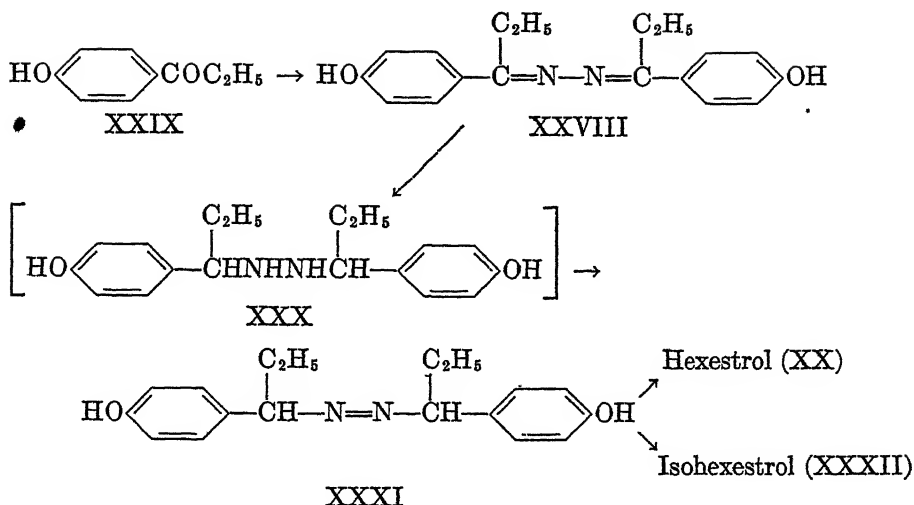
B. SYNTHESSES (METHODS OTHER THAN HYDROGENATION)

For didactic reasons direct syntheses of hexestrol, i.e., synthetic methods other than hydrogenation of stilbene derivatives, will be discussed here separately.

Simultaneously with the hydrogenation of dienestrol (XXI) to hexestrol by Campbell, Dodds, and Lawson (25), the same authors reported the first direct synthesis in the course of their work on the identification of hexestrol among the demethylation products of anethole. Anisaldazine (XXVI) was reacted with two moles of ethylmagnesium bromide to yield hexestrol dimethyl ether (XXVII).



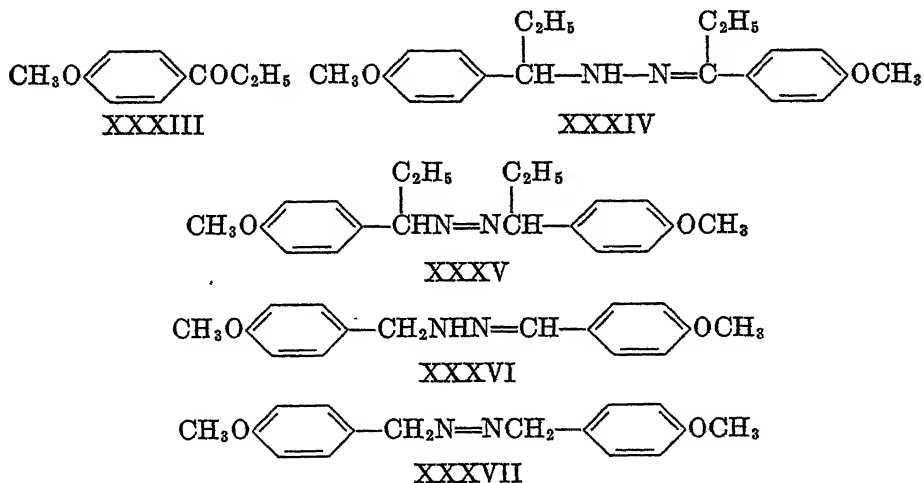
By demethylation hexestrol was obtained in poor over-all yield, but this synthesis confirmed the structure of hexestrol as 3,4-bis(*p*-hydroxyphenyl)hexane (XX). Bretschneider *et al.* (16, 17) later improved the yield of this synthesis (25); nevertheless the method remains without practical significance. Bretschneider *et al.* (16, 29) also developed another original synthesis with an over-all yield of 10 per cent. The ketazine (XXVIII) of *p*-hydroxypropiophenone (XXIX) is hydrogenated with palladium on charcoal to a tetrahydro derivative (XXX) (not isolated), which was oxidized with iodine, air, or simply by vacuum distillation (30) to a dihydro derivative (XXXI), possibly existing in two forms; on heating this compound above 120°C. nitrogen is lost to give a mixture of hexestrol and an optical isomer of the same, called isohexestrol (XXXII).



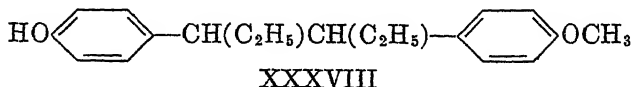
The success of this synthesis is interesting in view of the negative attempts of Linnell and Sharma (115) to remove nitrogen directly from the ketazine (XXVIII).

Foldi and Fodor (81, 160) in collaboration with Bretschneider applied the same method to the ketazine of *p*-methoxypropiphenone (XXXIII). The dihydroketazine (XXVIII) was isolated in two isomeric forms. Both isomers on thermal decomposition gave equal parts of hexestrol dimethyl ether and isohexestrol dimethyl ether.

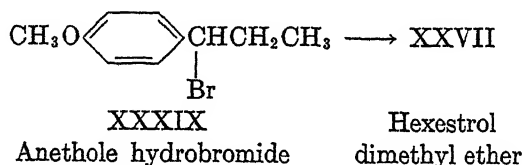
Fodor and Szarvas (79) investigated the absorption spectra of the two intermediate dihydroketazines, in order to decide between the two formulas (XXXIV and XXXV), by comparison with the spectra of anisal anisylhydrazone (XXXVI) and 4,4'-dimethoxy- α,α' -azotoluene (XXXVII).



The absorption spectra of the two isomeric dehydroketazines coincide with each other as well as with that of compound XXXVII, while compound XXXVI shows an entirely different spectrum. This excludes formula XXXIV, but no decision can be made between the possibility of either *cis-trans* isomerism or *meso-dl* isomerism. By simultaneous decomposition of a mixture of *p*-hydroxy- and *p*-methoxy-propiophenone dihydroketazines, the monomethyl ether (XXXVIII) of hexestrol has been prepared (16). This method is of considerable theoretical interest, representing one of the rare cases of an association of unsymmetrical radicals formed by decomposition of two different substances.

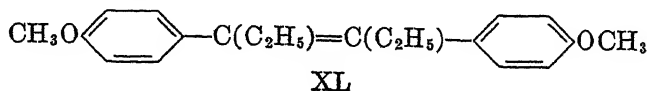


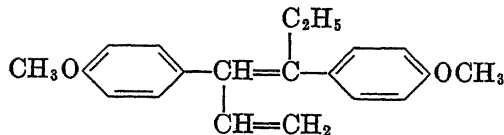
Peak and Short (13, 145, 190) synthesized hexestrol by a shorter method, starting from the known (124, 143) anethole hydrobromide (XXXIX) which is readily accessible from anethole (XIII) and hydrobromic acid. By the action of sodium, magnesium, aluminum, zinc, or the liquid alloy of potassium and sodium, two molecules undergo the Wurtz reaction and yield hexestrol dimethyl ether (XXVII).



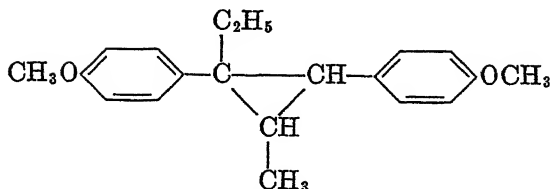
Instead of anethole hydrobromide, the hydrochloride or hydroiodide may be used and the reaction may be catalyzed by iodine, ethyl bromide, or Grignard reagent. Docken and Spielman (49) independently, though later, arrived at the identical method and reported an over-all yield of 10 to 15 per cent. Braker and Pribyl (15) obtained a United States patent on the same synthesis. This route was also followed independently by Bernstein and Wallis (11, 214), who prepared *p*-(α -bromopropyl)anisole (anethole hydrobromide) (XXXIX) in a longer, though inexpensive way from *p*-hydroxypropiofenone (XXIX) and then continued in essentially the same manner. Anethole hydrobromide has also been prepared (13) from anisaldehyde by reaction with ethylmagnesium bromide, followed by bromination with phosphorus tribromide.

Kharasch and Kleiman (99) effected the dehydrobromination of anethole hydrobromide by means of sodium amide in liquid ammonia. In this case an unsaturated dimethyl ether isomeric, but not identical, with stilbestrol dimethyl ether (XL) is obtained and is believed to be represented by either one of the two structural formulae XLI or XLII.



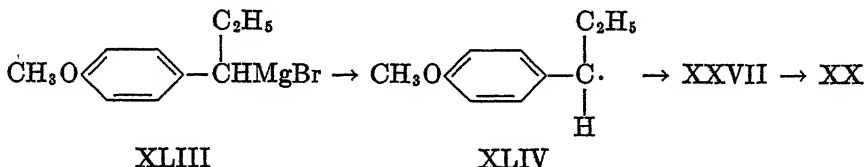


XLI



XLII

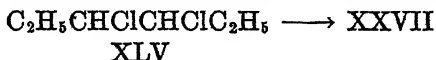
Hydrogenation with platinum black gives a theoretical yield of a hexestrol dimethyl ether (XXVII). The same authors (100) devised an even shorter synthesis with a 42 per cent over-all yield which may be superior to all others. By means of cobaltous chloride the Grignard compound (XLIII) of anethole hydrobromide is reduced to a free radical (XLIV), which dimerizes to hexestrol dimethyl ether (XXVII).



XLIII

XLIV

Of theoretical interest only is a synthesis found by Price and Mueller (154), who subjected 3,4-dichlorohexane (XLV) to a Friedel-Crafts reaction with anisole and obtained a very small yield of hexestrol dimethyl ether (XXVII).



XLV

C. OPTICAL ISOMERISM

Dodds, Goldberg, Lawson, and Robinson (59) obtained two isomeric forms of 3,4-bis(*p*-hydroxyphenyl)hexane (XX), one melting at 185°C., the other at 128°C. The relationship of these two isomers to stilbestrol and its isomer will be treated in a subsequent chapter. The higher melting form was called hexestrol by Campbell, Dodds, and Lawson (24), who also reported its superior estrogenic activity as compared with the lower melting form, called isohexestrol by Peak and Short (145). Wessely and Welleba (224, 225) reported that the purest preparations of hexestrol do not have a clear melting point at 186°C., and even after purification by way of the diacetate, hexestrol contains an impurity melting at 230°C. with decomposition. These authors succeeded in assigning the *meso*-form to hexestrol through resolution of isohexestrol into two

TABLE 2
Homologs of hexestrol

FORMULA $\begin{array}{c} \text{R} \quad \text{R}' \\ \quad \\ \text{HO}-\text{C}_6\text{H}_4-\text{CH}=\text{CH}-\text{C}_6\text{H}_4-\text{OH} \end{array}$		MELTING POINT	NAME	ESTROGENIC ACTIVITY IN RATS SUBCUTANEOUSLY (100 PER CENT RESPONSE, UNLESS OTHERWISE INDICATED)		REFERENCES
R	R'			Minimum effective dose	Rat units per gram	
		°C.		micrograms		
H	H	198-199	1,2-Bis(<i>p</i> -hydroxyphenyl)ethane	100,000		(64)
CH ₃	CH ₃	138-139 139-139.5 231-232	2,3-Bis(<i>p</i> -hydroxyphenyl)butane	0.5 10 1,000	2,000,000	(56) (1, 75a) (1)
C ₂ H ₅	C ₂ H ₅	186	3,4-Bis(<i>p</i> -hydroxyphenyl)hexane <i>Meso</i> -form ("hexestrol")	0.2 2.0	5,000,000	(24, 56) (223)
		129 130 80 80	Racemate ("isohexestrol") +Antipode -Antipode	1,000 500 -100 1,000 (40%)		(25) (223, 225) (225) (225)
C ₃ H ₇	C ₃ H ₇	165 b.p. 185/0.1 mm.	4,5-Bis(<i>p</i> -hydroxyphenyl)octane (a) (b)	5 100	200,000 10,000	(56) (56)
C ₁₈ H ₃₃	H	86-87	1,2-Bis(<i>p</i> -hydroxyphenyl)- octadecane	100,000	10	(56)

antipodes by means of α -bromocamphor- π -sulfonic acid. Equal parts of the two antipodes (m.p. 80°C., $[\alpha]_D^{17} = +17.7^\circ$ and -17.6° , respectively) mixed together showed the melting point, 129°C., of the racemate. The two antipodes and the racemate possess entirely different activities, the *d*-form being five times more active than the racemate, and ten to twenty times more active than the *l*-form (see table 2). The following point might illustrate the limitations in comparing estrogenic potencies, even those reported from the same laboratory. Unless the *l*-antipode should have an inhibitory effect on the activity of the *d*-form, an extremely remote possibility, it is not clear why the racemate should be less than one-half as active as the *d*-form.

Interesting rearrangements have been observed for the pair of dimethyl ethers; hexestrol dimethyl ether melts at 145–146°C. and isohexestrol dimethyl ether melts at 56°C. (see table 6).

Bretschneider *et al.* (15a, 16), while attempting unsuccessfully to dehydrogenate the isomeric hexestrols to stilbestrol derivatives, observed that both dimethyl ethers are attacked when heated in the presence of palladium on charcoal. Hexestrol dimethyl ether remains unchanged to the extent of 28 per cent, while the remainder is converted into a substance not yet identified. When isohexestrol dimethyl ether is treated under similar conditions 42 per cent is converted into hexestrol dimethyl ether, or 70 per cent on the basis of recovered unchanged material. The authors pointed out the practical significance of thus converting an estrogen of medium into one of highest potency.

Peak and Short (145) observed a 50 per cent conversion of the racemate into the *meso*-form and partial conversion in the reverse direction by heating to 300°C. in an atmosphere of hydrogen sulfide. Peak and Short remarked that "it is difficult to envisage the observed isomerizations except on the basis either of reversible dehydrogenation or of fission and of recombination." However this isomerization is reminiscent of the well-known partial racemization of tartaric acid, leading to an equilibrium between the *meso*-form and the racemate.

The assignment of the *meso*-form was confirmed by Carlisle and Crowfoot (27), who undertook a study of x-ray crystallographic measurements of a series of *p,p'*-substituted diphenylhexane derivatives. For *p,p'*-dihydroxy derivatives (hexestrol and isohexestrol), as well as for the 4,4'-diamino and the 4,4'-dicarbomethoxy derivatives, it could be shown that the higher melting isomers possess the molecular symmetry characteristic of the *meso*-form, while the lower melting forms are racemic.

IV. STILBESTROL

A. SYNTHESSES

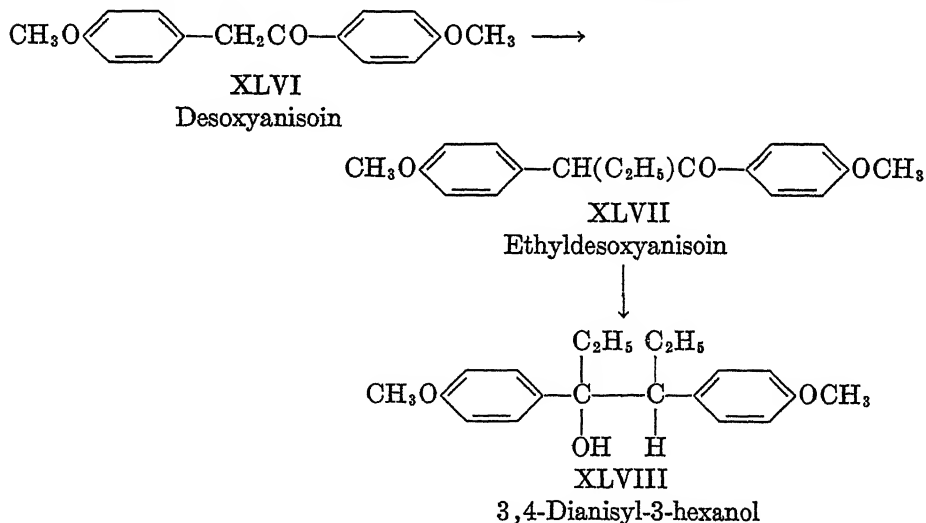
The first synthesis from desoxyanisoin (XLVI) was published in 1938 by Dodds, Goldberg, Lawson, and Robinson (58) simultaneously with the disclosure of the potency of stilbestrol. The relatively poor yield and the increasing demands for therapeutic use were incentives for many workers to develop new syntheses. The structure of stilbestrol lends itself to a large number of

synthetic approaches. Several among the syntheses represent definite advances over Dodds's original method and appear suitable for production on a larger scale. Other procedures are of only theoretical interest; nevertheless they demonstrate interesting relationships between the intermediates of various syntheses.

In the following discussion the synthetic methods have been broken down into types of approaches irrespective of their practical usefulness.

1. Syntheses from anisoin

Dodds and coworkers (58, 59) reacted desoxyanisoin (XLVI) with ethyl iodide and sodium ethoxide to give ethyldesoxyanisoin (XLVII). Introduction of the second ethyl group by a Grignard reaction resulted in one of the two racemic forms, m.p. 117°C., of 3,4-dianisyl-3-hexanol (XLVIII). The other isomeride, m.p. 85°C., was later isolated by Braker *et al.* (14) and by Wessely and coworkers (222). Dehydration of the carbinol (XLVIII) to a stilbene derivative was originally (58) effected by means of phosphorus tribromide, potassium bisulfate, or a mixture of acetic anhydride and acetyl chloride; later Kuwada *et al.* (107) used hydrochloric acid, and Wessely *et al.* (222) used potassium pyrosulfate. These reactions led to a mixture of the liquid *cis*- and the crystalline *trans*-forms of 4,4'-dimethoxy- α,β -diethylstilbene (XL).

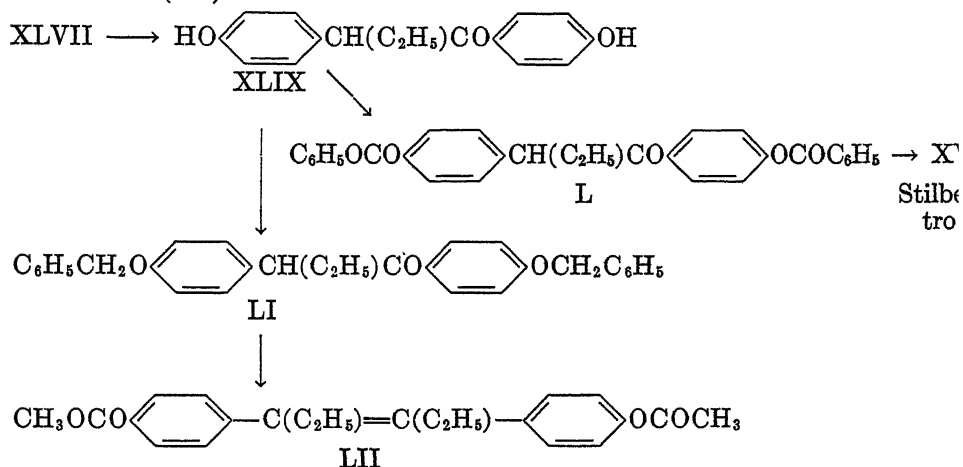


Subsequent studies, discussed later, provided the evidence for the respective assignment of configuration. Rohrmann (168a, 168b) found that iodine in various solvents or the complex of boron trichloride (or, preferably, the trifluoride) with ethyl ether reacts with the carbinol (XLVIII) in carbon tetrachloride to give almost exclusively the desired *trans*-form. Wilds and Biggerstaff (228a) obtained the same result with *p*-toluenesulfonic acid.

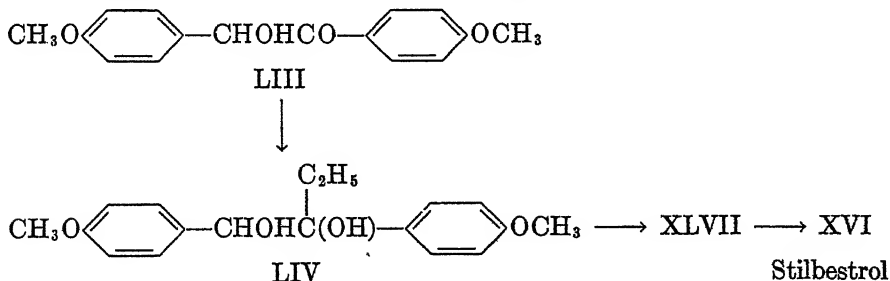
The last step in the synthesis, demethylation of the methoxyl groups, caused considerable difficulties. Dodds *et al.* (58) used potassium hydroxide and ethanol at 228°C., Zajik and Wessely (230) Grignard reagent at elevated temperatures,

and Corse (37, 68a), hydroxides of sodium, potassium, or lithium dissolved in ethylene, diethylene, dipropylene, or triethylene glycols at 190–235°C. Rubin *et al.* (170) found that the demethylation by sodium or sodium hydroxide in boiling diethylene glycol monoethyl ether (Carbitol) yields a mixture of stilbestrol and its monomethyl ether, with the latter predominating.

Dodds *et al.* (59) further reported two modifications of their original synthesis. The difficult demethylation of the phenolic methoxyl groups is effected at an earlier stage by treating ethyldesoxyanisoin (XLVII) with hydriodic acid to give 4,4'-dihydroxy- α -ethyldesoxybenzoin (XLIX). Therefrom the dibenzoic ester (L) was prepared which was reacted with 5 moles of ethyl Grignard reagent to give stilbestrol (XVI) directly. The other modification is by way of the dibenzyl ether (LI), followed by Grignard reaction and acetylation to stilbestrol diacetate (LII).

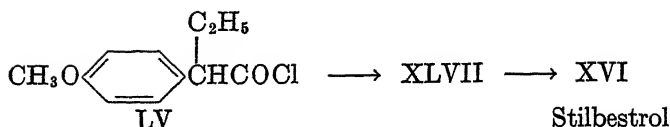


Kuwada and Sasagawa (106) modified the original synthesis by introducing the first ethyl group into anisoin (LIII) by means of a Grignard reaction to give 3,4-bis(*p*-anisyl)-3,4-butanediol (LXIV); dehydration with sulfuric acid led to ethyldesoxyanisoin (XLVII), the key intermediate of Dodds's synthesis.



2. Introduction of anisyl group by Friedel-Crafts reaction

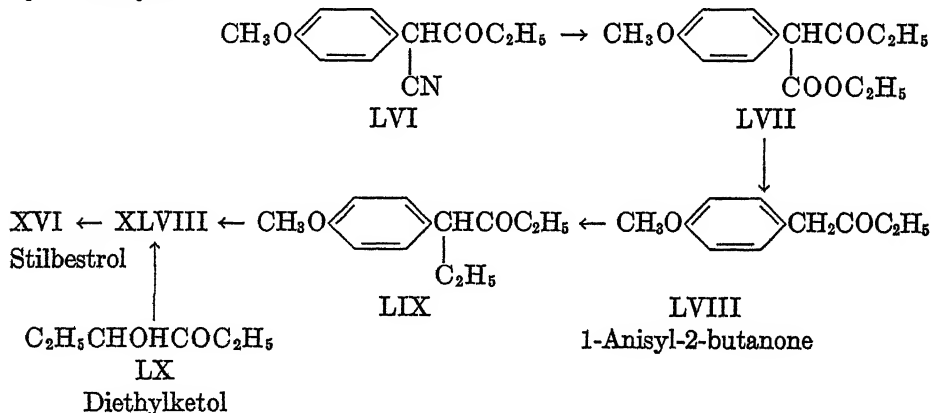
The same intermediate (XLVII) of the preceding syntheses was obtained by Andersag and Salzer (2) from α -anisyl- α -ethylacetyl chloride (LV), by a Friedel-Crafts reaction with anisole.



While this Friedel-Crafts synthesis has not yet been reported in detail, Wilds and Biggerstaff (228a) independently arrived at essentially the same method. These authors obtained the acid chloride (LV) from the readily available α -phenylbutyric acid by a series of standard reactions.

3. Introduction of anisyl group by Grignard reaction

Wessely and coworkers (222) devised a stilbestrol synthesis starting from *p*-methoxybenzyl cyanide, which was subjected to a Claisen condensation to give α -propionyl-*p*-methoxybenzyl cyanide (LVI) and, by way of the imido ether, ethyl α -propionyl- α -*p*-methoxyphenyl acetate (LVII). After hydrolysis and decarboxylation to 1-anisyl-2-butanone (LVIII), the second ethyl group was introduced by means of ethyl iodide and sodium ethoxide, resulting in 3-(*p*-anisyl)-4-hexanone (LIX). The Grignard reaction of the hexanol with anisylmagnesium bromide gave predominantly the carbinol (XLVIII), m.p. 117°C., described by Dodds *et al.* (58, 59), in addition to a new isomer melting at 85°C. The synthesis was completed by dehydration to stilbestrol dimethyl ether, followed by demethylation.



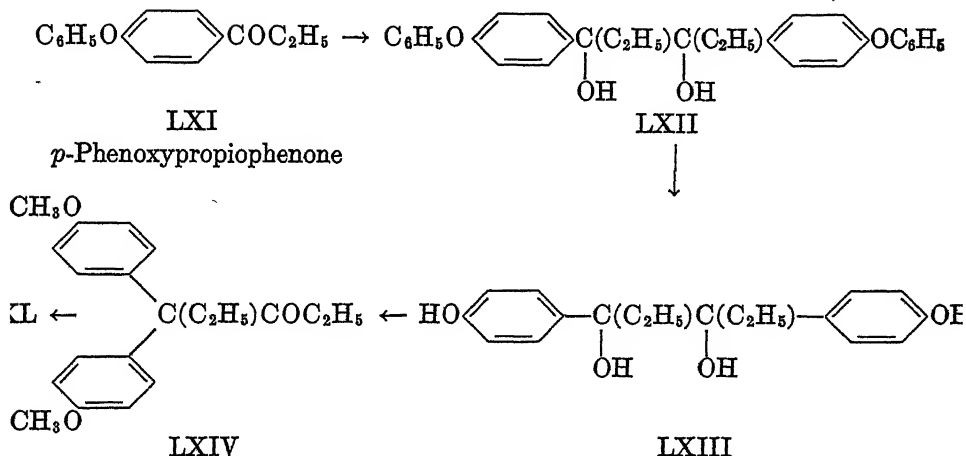
The same synthesis is a subject of a patent claim by Fieser and Christiansen (78). Kuwada *et al.* (107) introduced both anisyl groups by Grignard reactions into diethylketol (LX), arriving at the carbinol (XLVIII).

4. Syntheses involving retro-pinacolin rearrangements

A number of syntheses are based on the intermediate formation of asymmetric α, α -dianisylethane derivatives, which are subject to the retro-pinacolin rearrangement to stilbene derivatives. Part of this work is based on investigations by Orekhoff (142) on the corresponding phenyl analogs.

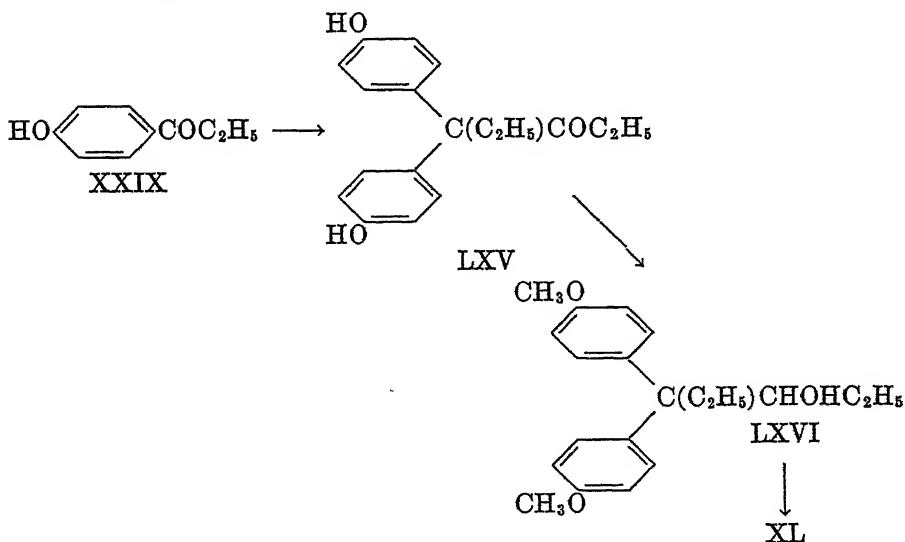
Hobday and Short (91) reduced 4-phenoxypropiophenone (LXI) electrolytically and dephenylated the resulting pinacol (LXII) to the lower melting (m.p.

94–95°C.) of the two isomeric forms of 3,4-bis(*p*-hydroxyphenyl)-3,4-hexanediol (LXIII).



The pinacol (LXIII) undergoes the pinacolin rearrangement to give, after methylation, 3,3-bis(*p*-anisyl)-4-hexanone (LXIV). On reduction of this compound with sodium and amyl alcohol retro-pinacolin rearrangement takes place to give the dimethyl ether (XL) of stilbestrol.

Wessely *et al.* (222) effected a similar series of reactions starting from *p*-hydroxypropiphenone (XXIX). In this case the other isomeric 3,4-bis(*p*-hydroxyphenyl)-3,4-hexanediol (LXIII), m.p. 204–206°C., was obtained as an intermediate. (Dehydration by means of a mixture of acetyl chloride and acetic anhydride gives a diene, dienestrol (XXI), to be discussed later). Potassium bisulfate, potassium persulfate, or acetic anhydride cause pinacolin rearrangement to 3,3-bis(*p*-hydroxyphenyl)-4-hexanone (LXV), also obtained by Adler *et al.* (1) by reacting LXIII with hydriodic acid and phosphorus.



After conversion of LXV to the methyl ether (LXIV), the former authors obtained the intermediate pinacolin alcohol (LXVI), which then underwent dehydration and retro-pinacolin rearrangement to stilbestrol dimethyl ether (XL).

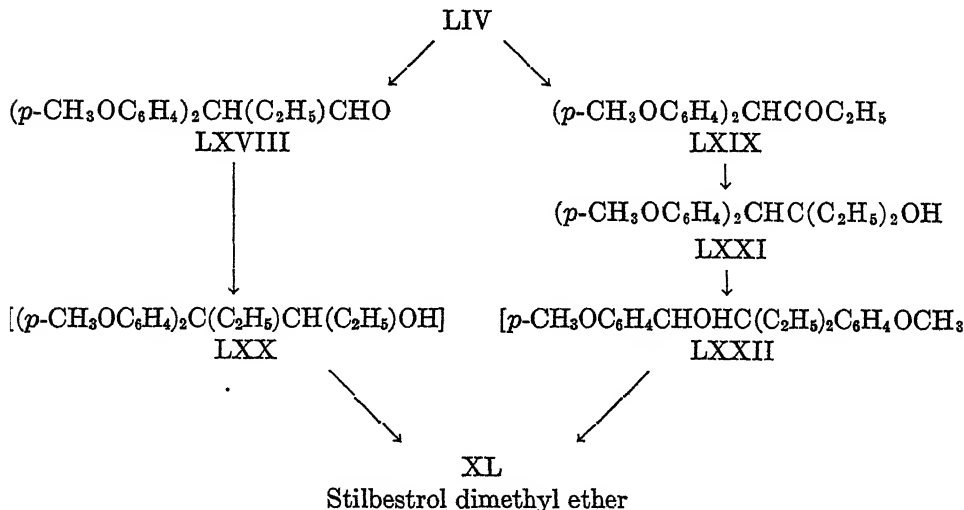
Surprisingly enough, Tendick (210a, 210b) found that pinacols of type LXIII, with free or acylated phenolic hydroxyl groups, do not undergo the pinacolin rearrangement when treated with strong mineral acids. By this method the author obtained the epoxy derivatives of type XCII, which were then reduced with sodium and alcohol to 3,4-bis(*p*-hydroxyphenyl)-3-hexanol or its phenol esters.

Returning briefly to the two isomeric forms of the pinacol (LXIII), it should be mentioned that the higher melting isomer, m.p. 204–206°C., was first obtained by Dodds and coworkers (58, 59, 60) in the course of their synthesis of dienestrol, by reduction of *p*-hydroxypropiophenone (XXIX) with aluminum amalgam in moist ether; Robinson and Resuggan (161) claimed a better yield by electrolytic reduction. According to Hobday and Short (91), either one of these two methods gives both isomeric forms of the pinacol (LXIII). Finally, the last authors obtained the higher melting form from the corresponding dimethyl ether by reaction of two moles of anisylmagnesium bromide with dipropionyl (LXVII).

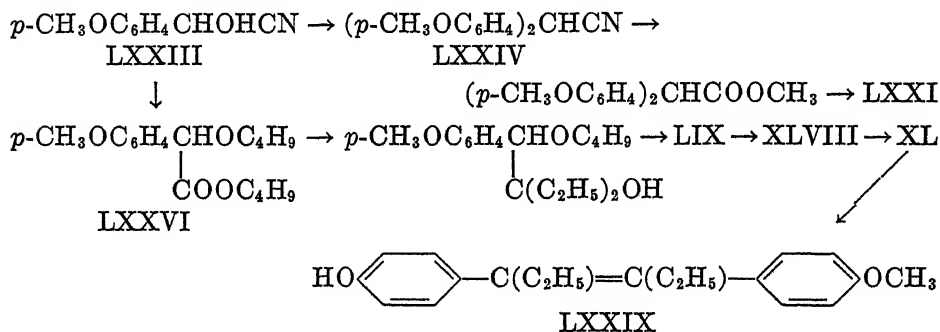


LXVII

Peteri (147, 148) contributed three syntheses of stilbestrol involving molecular rearrangements. The first two have as a common starting point 3,4-bis(*p*-anisyl)-3,4-butanediol (LIV), encountered earlier in another synthesis (106). Depending on the dehydrating conditions this substance may rearrange in two ways to α,α -dianisylbutyraldehyde (LXVIII) and 1,1-anisyl-2-butanone (LXIX). Both are reacted with ethylmagnesium bromide to give LXX and LXXI, and are then subjected to dehydration and a second rearrangement of the retro-pinacolin type encountered earlier.

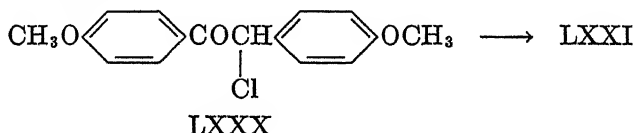


The rearrangement of 1,1-bis(*p*-anisyl)-2-ethyl-2-butanol (LXXI) over the hypothetical intermediate (LXXII) by means of phosphorus oxychloride in toluene occurs in 32 per cent yield. The intermediate (LXXI) may be prepared by a third synthesis devised by this worker. Starting from anisaldehyde cyanohydrin (LXXIII), the second anisyl group is introduced by means of sulfuric acid to give α,α -dianisylacetonitrile (LXXIV). After hydrolysis and esterification to LXXV, substitution with both ethyl groups is effected by a Grignard reaction, resulting in LXXI.



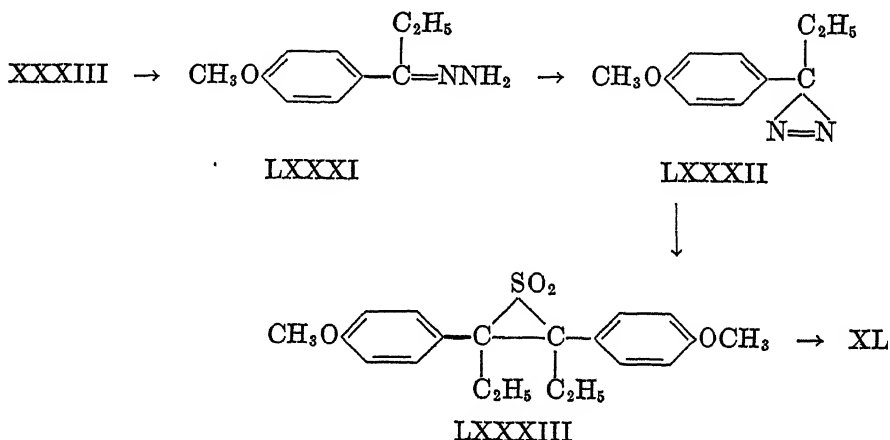
Rubin *et al.* (170) reported another synthesis involving the same starting material (LXXIII) and also a retro-pinacolin rearrangement with alkyl migration.

Anisaldehyde is converted to the cyanohydrin (LXXIII) and by reaction with butanol yields butyl α -anisyl- α -butoxyacetate (LXXVI). In the second step two ethyl groups are introduced by means of ethylmagnesium chloride, but the intermediate carbinol (LXXVII) need not be isolated and is dehydrated under rearrangement to 3-(*p*-anisyl)-4-hexanone (LIX), encountered previously in other syntheses (78, 222). In variation to the latter, Rubin *et al.* preferred to substitute with the second anisyl group by means of 4-chloroanisole and sodium, resulting in the carbinol (XLVIII) which is dehydrated, without isolation, to a mixture of stilbestrol dimethyl ether (XL) and its *cis*-isomer. The third step is completed by isomerization of the *cis*-isomer in the presence of iodine and ferric chloride, and the fourth step comprises demethylation by means of sodium or sodium hydroxide in boiling Carbitol; under these conditions the larger part is converted into stilbestrol monomethyl ether (LXXIX) desired in this instance. The over-all yield from anisaldehyde is 12 per cent for the monomethyl ether in addition to 9 per cent for stilbestrol. Foldi and Demjen (80) reacted chlorodesoxyanisoin (LXXX), also described by Dodds (56), with ethylmagnesium bromide and obtained the carbinol (LXXI), owing to change of position of an alkyl and an aryl group.

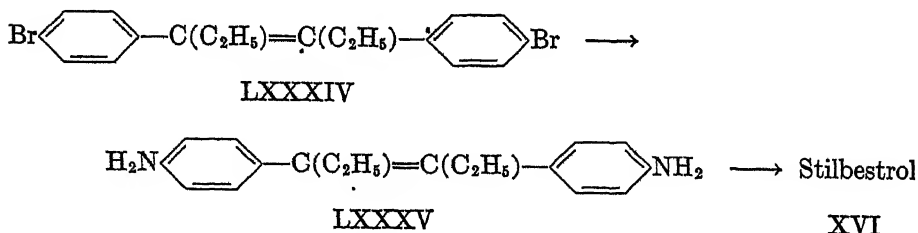


5. Syntheses involving miscellaneous rearrangements

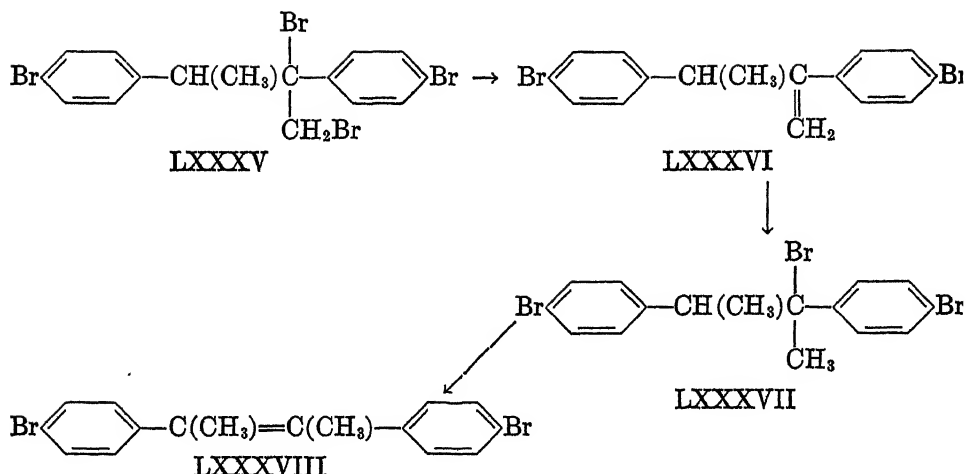
Vargha (159, 212, 213) developed an interesting synthesis with an over-all yield of 25 per cent from *p*-methoxypropiophenone (XXXIII) to stilbestrol dimethyl ether. The starting material is transformed into the hydrazone (LXXXI) and oxidized with mercuric oxide to anisylethyldiazomethane (LXXXII), which need not be isolated. It easily decomposes under partial loss of nitrogen to the ketazine (XXXV) obtained by Foldi and Fodor (81), but with sulfur dioxide in petroleum ether it mainly reacts under total loss of nitrogen to give the sulfone (LXXXIII) and by thermal decomposition of the latter, stilbestrol dimethyl ether (XL) is obtained.



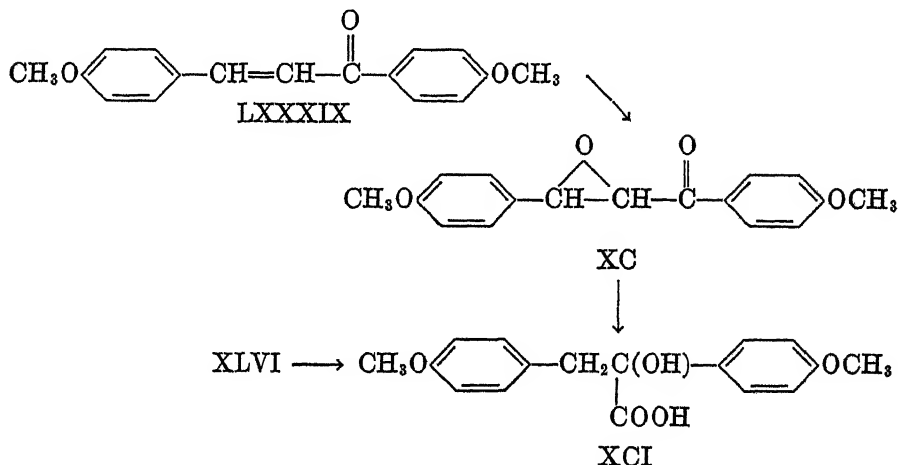
Vargha and Kovacs (213) used this method for the synthesis of the 4,4'-dibromo analog (LXXXIV) of stilbestrol; by the action of cuprous iodide and ammonium hydroxide the bromo compound was converted to the 4,4'-diamino analog (LXXXV) and by a Sandmeyer reaction to stilbestrol; the latter could not be directly obtained from the dibromo compound (LXXXIV).



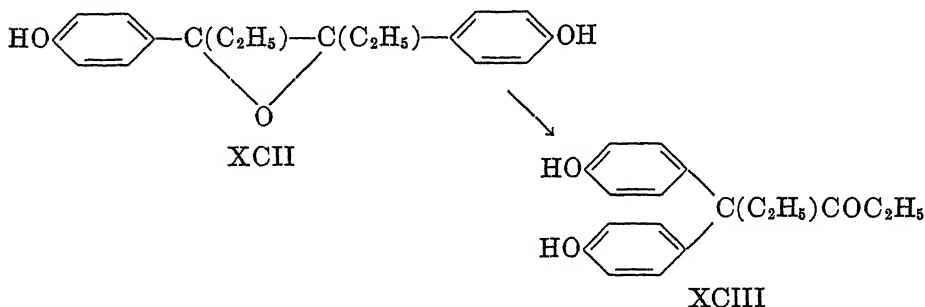
Similar bromo derivatives are accessible by still another method. Barber (9, 10) prepared 4,4'-dibromo- α,β -dimethylstilbene (LXXXVIII) by way of the compounds LXXXV to LXXXVII.



Kharasch and Kleiman (99) devised the shortest synthesis of stilbestrol with the impressive over-all yield of 22 per cent, including the final demethylation, starting from anethole hydrobromide (XXXIX). Under the influence of sodium amide in liquid ammonia two molecules couple with loss of bromine. The resulting product, m.p. 120.5°C., is isomeric but not identical with either stilbestrol dimethyl ether or its *cis*-isomer. It was mentioned before that either one of the two structures XLI and XLII has been assigned to the product. During demethylation rearrangement takes place, resulting in a mixture of stilbestrol and an unidentified oil which by repeated demethylation is also converted to stilbestrol. Rohrmann, Jones, and Shonle (168, 169) applied some previously known reactions of chalcones to a new synthesis of the important intermediate desoxyanisoin (XLVI). Anisyl *p*-methoxystyryl ketone (LXXXIX) is oxidized with hydrogen peroxide and alkali to the epoxy derivative (XC), which is rearranged with alkali to *p*-anisyl(*p*-methoxybenzyl)glycolic acid (XCI). By oxidation with red lead and simultaneous decarboxylation, desoxyanisoin (XLVI) is obtained in 50 to 70 per cent over-all yield.



A similar rearrangement had been described earlier by Wessely *et al.* (98, 222); the epoxy derivative (XCII) of stilbestrol on heating rearranges to 3,3-bis(*p*-hydroxyphenyl)-4-hexanone (XCIII).

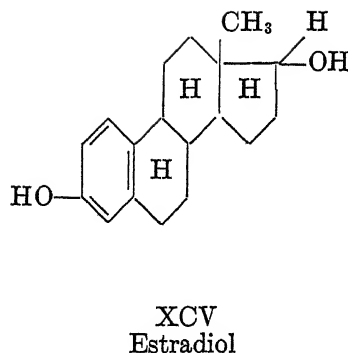
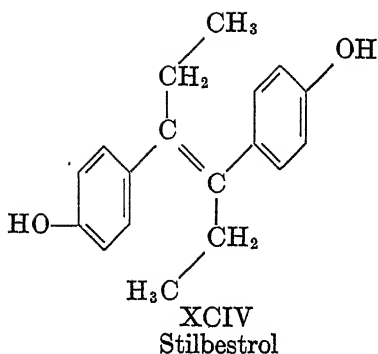


B. *Cis-trans* ISOMERISM OF STILBESTROL

The theory requires two *cis-trans* isomers for a compound possessing the structure (XVI) of stilbestrol, and the problem of assigning the proper configuration to the two isomers has prompted much theoretical and experimental work. In the course of their synthesis of stilbestrol Dodds *et al.* (58, 59) dehydrated 3,4-dianisyl-3-hexanol (XLVIII) to obtain two isomeric forms (XL): one crystalline, m.p. 123–124°C., after demethylation yielding stilbestrol, m.p. 171°C.; the other an oil, b.p. 175–178°C. at 0.74 mm., on demethylation yielding ψ -stilbestrol, m.p. 141°C. (later 151°C.). The oily dimethyl ether is gradually transformed into the crystallized isomer, stilbestrol dimethyl ether, when exposed to sunlight and especially in the presence of iodine. For this Rubin *et al.* (170) used iodine with ferric chloride, antimony pentachloride, aluminum trichloride, or boron trifluoride while heating at 140°C. Serini and Steinruck (188) found that the rearrangement during the demethylation by means of alcoholic alkali is favored at temperatures above 200°C., in the presence of iodine at 180°C., or just by shaking at room temperature with palladium.

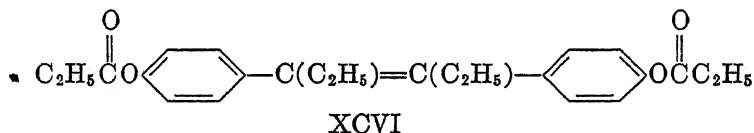
According to Wessely and Welleba (225) the reverse transformation could not be effected. Yet this finding was not conclusive as an argument for assigning the *cis* or *trans* configuration to stilbestrol, because under similar conditions, *cis*- α,β -dimethylstilbene also cannot be rearranged to its *trans*-isomer. Wessely (219) further noted that higher thermostability alone is no proof for the *trans* configuration, because several instances (68, 210) are known where the *cis*-form of certain ethylene derivatives proved to be the more stable.

Dodds, Robinson, and their coworkers (58, 59) reported that ψ -stilbestrol has only a fraction of the estrogenic activity of stilbestrol. These authors held that the difference in activity was strong evidence in favor of the *cis* configuration for ψ -stilbestrol, reasoning that stilbestrol, as the more active isomer, should have the *trans* configuration more closely related to estradiol. This resemblance was underlined by writing the structural formula (XCIV) for stilbestrol in a manner resembling that of estradiol (XCV).



For a number of years no unequivocal proof could be provided for the *trans* configuration of stilbestrol. This was mainly due to the difficulties encountered in obtaining pure ψ -stilbestrol. Consequently the conditions were unfavorable for a direct and conclusive comparison of the physicochemical characteristics of the two isomers, such as dipole measurements (219), absorption spectra, or crystallographic x-ray measurements.

Only recently reports have come forth from three different laboratories regarding the purification of ψ -stilbestrol and its dipropionate. Peteri (146) reacted stilbestrol with propionic anhydride or pyridine and, in addition to the previously known (57) stilbestrol dipropionate (XCVI), m.p. 105–106°C., obtained from the mother liquors an isomeric dipropionate, m.p. 71–72°C. Both dipropionates on hydrolysis give exclusively stilbestrol; this result may be explained by the extraordinary lability of ψ -stilbestrol.



Wessely, Bauer, and Kerschbaum (220) confirmed these results. However, these workers indicated that propionylation of the purest stilbestrol does not give rise to the *cis*-isomer, while the product of mild hydrolysis of the dipropionate, m.p. 71–72°C. (79°C. for the purest preparation), on renewed propionylation gives a mixture of both isomeric propionates.

Walton and Brownlee (215) made the most thorough investigation of the propionic esters and confirmed Peteri's results. Furthermore, these authors succeeded in a further purification of a ψ -stilbestrol preparation with m.p. 141°C. by extended fractionation from benzene, resulting in stilbestrol, m.p. 171°C. (needles), and ψ -stilbestrol, m.p. 151°C. (hexagonal tablets). This preparation is believed to be pure, as evidenced by the melting-point diagram which shows that the impure preparation of Dodds *et al.* (59), m.p. 141°C., was approximately a eutectic mixture containing 60 per cent ψ -stilbestrol and 40 per cent stilbestrol. After a preliminary study, Dodds and Robinson agreed (215) with this conclusion.

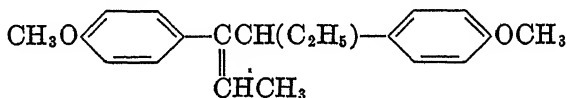
The situation is complicated by the fact that Walton and Brownlee esterified pure ψ -stilbestrol, m.p. 151°C., and obtained an oily dipropionate. This oily dipropionate on alkaline hydrolysis gives a theoretical yield of ψ -stilbestrol, and in the opinion of the authors the oil may prove to be a mixture. However, it is difficult to see why such a mixture should hydrolyze exclusively to ψ -stilbestrol, while either one of the two crystalline propionates yields exclusively stilbestrol. The interrelationship of these compounds is shown in table 3. By direct comparison, Walton and Brownlee determined the relative estrogenic activities:

Stilbestrol: ψ -stilbestrol = 14:1

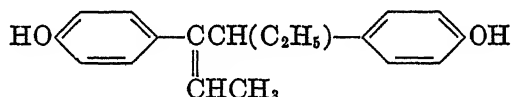
Stilbestrol dipropionate: ψ -stilbestrol dipropionate = 600:1

Stilbestrol dipropionate: oily ψ -stilbestrol dipropionate = 16:1

In the following way Wessely *et al.* (220) provided good evidence for the location of the double bond in the crystalline ψ -stilbestrol dipropionate. This group of investigators had previously (222, 223) shown that dehydration of the carbinol (XLVIII) by means of potassium acid sulfate results, in addition to the dimethyl ethers of stilbestrol and ψ -stilbestrol, in a pair of two structural isomers (XCVII): the one an oil, the other crystalline, m.p. 50°C.



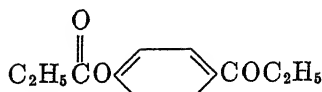
XCVII



XCVIII

The structure of these new isomers was proved by ozonization to ethylde-soxyanisoin (XLVII). With iodine as a catalyst both isomers rearrange to stilbestrol dimethyl ether (XL). The two compounds are *cis-trans* isomers with the configuration not determined, both being racemic regarding the asymmetric carbon atom. The dimethyl ethers (XCVII) are demethylated to the two corresponding forms of 3,4-bis(*p*-hydroxyphenyl)-2-hexene (XCVIII), m.p. 43.5°C. and 053°C., respectively. It is worth noting that one of these is highly potent, while the other has only medium activity. A resolution of these racemates into the optical antipodes has not been reported. Wessely (220) found that both forms of 3,4-bis(*p*-hydroxyphenyl)-2-hexene give oily dipropionates not identical with the crystallized dipropionates of stilbestrol and ψ -stilbestrol. This finding refutes a statement by Jones (96) that the hexene derivative, m.p. 053°C., may be identical with ψ -stilbestrol, m.p. 151°C. It remains to be seen how the oily dipropionate obtained from ψ -stilbestrol is related to the others or if it is identical with one of the dipropionates prepared from the hexene (XCVIII).

These results make it likely that the crystalline ψ -stilbestrol dipropionate is a stilbene derivative and thus the *cis*-isomer of stilbestrol. This was further corroborated by Wessely *et al.* (37), who ozonized the crystalline ψ -stilbestrol dipropionate, and obtained *p*-propoxypropiophenone (XCIX).



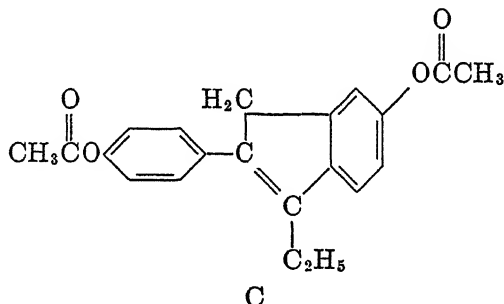
XCIX

p-Propoxypropionophenone

It was concluded that the starting material must have been an ester of the *cis*-isomer of stilbestrol, because fissure occurred in the same position as with stilbestrol, which was ozonized (222) to *p*-hydroxypropionophenone (XXIX).

Wessely and Welleba (224, 226) employed hydrogenation methods to prove the *trans* configuration of stilbestrol. The results will be discussed in a subsequent section.

The direct comparison of the ultraviolet-absorption spectra of stilbestrol and its isomer has not yet been possible, but the spectrum of stilbestrol alone is of interest. Solmssen (127) compared the spectra of the diacetate of stilbestrol and of 2-(*p*-acetoxyphenyl)-3-ethyl-6-acetoxy-2,3-indene (C).



This substituted phenylindene derivative has a spectrum almost identical with that of *trans*-stilbene and of the unsubstituted 2-phenylindene, according to Wiegand and Merkel (143), while stilbestrol has a quite different spectrum. It now appears that this disagreement of the spectra is due to the α,β -alkyl substitution of the stilbene double bond. The spectrum of stilbestrol was found to be very similar to that of α,β -diethylstilbene (136) and of *trans*- α,β -dimethylstilbene according to Arends (3), and in accordance with Kuwada and Sasagawa (106) it must be concluded that the *trans* configuration of stilbestrol is not in disagreement with its absorption spectrum. There is other strong evidence in favor of this configuration. Giacomello and Bianchi (86) studied the crystallographic constants of stilbestrol and its dimethyl ether. According to these authors the Patterson projection on the plane AC conforms with the *trans* structure of the molecule. Carlisle and Crowfoot (27) concluded from their study of crystallographic measurements that the general arrangement of the groups around the central double bond is that expected for the *trans* configuration in stilbestrol and stilbestrol dipropionate (m.p. 104°C.). However, the authors added, this was clearly established only in the case of the dipropionate, while in the other case a more detailed x-ray crystallographic analysis might reveal molecular symmetry not apparent from the preliminary measurements.

C. ANALYTICAL METHODS FOR STILBESTROL DETERMINATION

The commercial production of stilbestrol made it desirable to develop chemical and physical analytical procedures.

Dingemans (45, 46, 47) utilized the fuchsin-red color developed with antimony trichloride for the colorimetric determination of stilbestrol.

According to Huf and Widmann (93) this method is suitable for pure solutions only and for samples not larger than 2.5 micrograms. These workers developed a new method where stilbestrol is coupled with diazobenzenesulfonic acid and the resulting yellow-red coloration determined colorimetrically, with linear concentration-extinction relations in the range of 50–250 micrograms per cubic centimeter.

Tubis and Bloom (211), as well as Dracass and Foster (66), developed the phenol reaction of Folin-Ciocaltu as a reliable method which was adopted by the Council of Pharmacy and Medicine for New and Nonofficial Remedies (38). The method is based on colorimetric or photometric examination of stable blue tungstic oxides due to reduction of labile phosphomolybdic phosphotungstic acids by phenolic hydroxyls. A straight-line graph was obtained for amounts of 0.2 to 0.8 mg. of stilbestrol.

Another method adopted by the Council for New and Nonofficial Remedies (38) is that of Sondern and Burson (195, 196), based on the bromometric titration of phenols and reminiscent of brominations in the stilbene series by Linnell and Shaikmahamud (112). The method requires the strict observance of specified conditions, because 6 or 8 moles of bromine are consumed, depending on the temperature; the method is suitable for amounts from 1 to 40 mg. of stilbestrol.

A qualitative assay method recommended by the Council for New and Nonofficial Remedies (38) is based on the acetylation of stilbestrol and examination of the refractive index of the crystalline diacetate under the polarizing microscope.

The conversion of stilbestrol into the diacetate and its gravimetric determination have been adopted as a quantitative method in the United States *Pharmacopoeia* (149). It may be questioned if this method matches the accuracy of some of the quantitative methods discussed earlier.

Dechene (41) proposed the application of the xanthoprotein reaction; for amounts of 0.25 to 1.75 mg. of stilbestrol the method may be used with an error of ± 1 per cent, but only when stilbestrol is the sole phenyl derivative present.

Elvidge (69, 70) used the ultraviolet-absorption spectra for the quantitative determination of stilbestrol and its derivatives. Della Croce (42) has described several qualitative color reactions for stilbestrol.

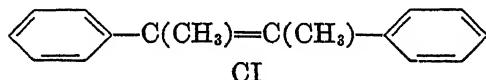
D. HYDROGENATION OF STILBESTROL

1. *Hydrogenation of aliphatic double bond*

The hydrogenation of stilbestrol and its derivatives reveals a rather involved relationship between members of the unsaturated and saturated series, as represented in table 3.

It is apparent that *cis* addition of hydrogen does not occur uniformly. Thus, there are many exceptions to the generally accepted conversion of derivatives of *cis*-stilbene into the *meso*-forms, and of *trans*-stilbene into the racemic forms of the hydrogenation products. It is also possible that primarily *cis* addition does take place but is followed by partial racemization of the type discussed earlier. In view of the fact that stilbestrol and its dimethyl ether have been hydrogenated to hexestrol as well as to isohexestrol, it may be questioned if it is sound to use results of hydrogenation experiments for deducing the *cis* or *trans* configuration of stilbestrol. Nevertheless, the conclusions drawn from these experiments have mostly been confirmed by various other means, discussed earlier.

Wessely and Welleba (224, 225) showed that the known *cis-trans* pair of α,β -dimethylstilbene (CI) is reduced almost exclusively by *cis* addition of hydrogen.



Under identical conditions (palladium sponge, acetic acid, 18°C., atmospheric pressure) stilbestrol and its dimethyl ether were also hydrogenated with the following comparative results:

α,β -Dimethylstilbene <i>trans</i> (m.p. 107°C.)	<i>cis</i> (m.p. 67°C.)	Stilbestrol	Stilbestrol dimethyl ether
↓	↓	↓	↓
98% racemic	99% <i>meso</i>	88% isohexestrol (<i>d,l</i>) 12% hexestrol (<i>meso</i>)	97% racemic 3,4- dianisylhexane
β,γ-diphenylbutane			

Therefrom it was concluded that stilbestrol and its dimethyl ether have the *trans* configuration. However, the formation of 12 per cent of the *meso*-form indicates that not qualitative but only quantitative differences exist in the formation of hydrogenation products, throwing some doubt on the validity of this deduction.

Wessely and Welleba (226) further approached the problem of the *cis* or *trans* configuration of stilbestrol by still another method based on hydrogenation products. Various *trans*-stilbenes were found to give mixed crystals with the *meso*-forms of the corresponding hydrogenation products, while they give a eutectic mixture with the racemic forms. The *trans* configuration of stilbestrol dimethyl ether was concluded from the experimental finding that this compound gives mixed crystals with hexestrol, but gives a eutectic mixture with isohexestrol.

According to Dodds *et al.* (56, 59), stilbestrol and ψ -stilbestrol with palladized charcoal in acetone give exclusively hexestrol dimethyl ether. On the other hand, at slightly elevated temperature, the same authors obtained, from the mixture of dimethyl ethers of stilbestrol and ψ -stilbestrol, a mixture of the dimethyl ethers of hexestrol and isohexestrol. Campbell *et al.* (25) obtained hexestrol also, as well as isohexestrol, by the hydrogenation of stilbestrol.

Wessely and coworkers (98, 225) insist that stilbestrol dimethyl ether cannot be hydrogenated to hexestrol dimethyl ether, while this latter is obtained from ψ -stilbestrol dimethyl ether in quantitative yield. According to these authors the crude reaction mixture from the dehydration of 3,4-bis(*p*-anisyl)-3-hexanol (XLVIII) is a useful starting material for the preparation of hexestrol. It is obtained after removal of the larger part of stilbestrol dimethyl ether, followed by hydrogenation and demethylation of the residue.

These last results are in accord with those of Docken and Spielman (49), who hydrogenated stilbestrol to isohexestrol in quantitative yield by the use of palladium, copper chromite, or Raney nickel catalyst.

Schwenk *et al.* (182) confirmed the quantitative production of isohexestrol from stilbestrol by hydrogenation with Raney nickel. By the more protracted reduction method developed by these workers—digesting nickel aluminum alloy in alkaline solution at atmospheric pressure—stilbestrol gave a 30 per cent yield of hexestrol and a 50 per cent yield of isohexestrol.

Hoehn and Ungnade (92) hydrogenated with Raney nickel at pressures up to 5000 lb. The dimethyl ether of stilbestrol gave isohexestrol dimethyl ether as the only product. Hydrogenation of stilbestrol monomethyl ether (LXXIX) under similar conditions gave a mixture of an alkali-soluble compound which on demethylation yielded hexestrol, and an alkali-insoluble compound believed to be hexestrol monomethyl ether (XXXVIII), described earlier by Bretschneider (16).

The hydrogenation of dienestrol (XXI) to hexestrol, in almost quantitative yield, has been described by Campbell, Dodds, and Lawson (25).

Wessely and Kleedorfer (223) found that, on hydrogenation with palladium, both isomeric forms of 3,4-bis(*p*-anisyl)-2-hexene (XCVII) give a 50 to 60 per cent yield of hexestrol dimethyl ether. The same authors (98) hydrogenated the epoxy derivative (XCII) of stilbestrol to a mixture of products containing 30 per cent hexestrol dimethyl ether. Jung (97) obtained a German patent for the hydrogenation of 3,4-bis(*p*-hydroxyphenyl)-3-hexanol to hexestrol. Unfortunately no experimental details of this method are available at present.

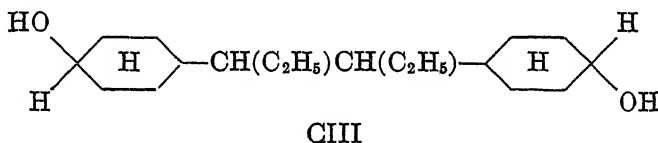
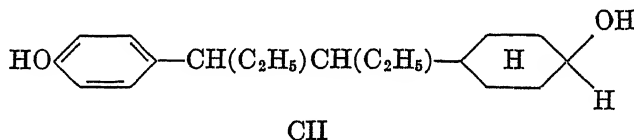
Peteri (146) hydrogenated stilbestrol dipropionate to the oily isohexestrol dipropionate, which was then hydrolyzed to isohexestrol. Correspondingly, ψ -stilbestrol dipropionate gave the crystalline hexestrol dipropionate and, after hydrolysis, hexestrol.

On the other hand, Walton and Brownlee (215) obtained hexestrol dipropionate from the crystalline dipropionates of both stilbestrol and ψ -stilbestrol. This again might be due to partial racemization of isohexestrol dipropionate or rearrangement of the unreduced stilbestrol dipropionate to ψ -stilbestrol dipropionate, because according to Wessely *et al.* (220) ψ -stilbestrol dipropionate readily isomerizes to stilbestrol dipropionate.

According to a Hungarian patent claim (160), hexestrol may be obtained from stilbestrol by hydrogenation of its esters in acetone, followed by saponification of the hydrogenation products.

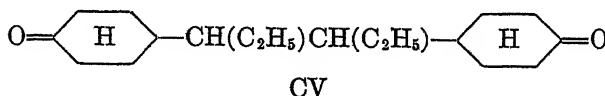
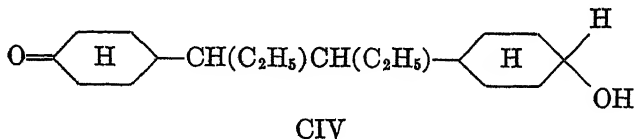
2. Hydrogenation of aromatic rings

Ruggli and Businger (172) made an unsuccessful attempt to synthesize hexahydrostilbene derivatives containing one cyclohexyl ring and thus more closely resembling estradiol. This objective could hardly be attained by hydrogenation methods, as no catalyst is as yet available for the selective hydrogenation of an aromatic ring while leaving an ethylene bond intact. After it had been shown that the ethylene bond is not essential for the attainment of highest potency, the preservation of this bond during reduction of one aromatic ring lost part of its interest. Hoehn and Ungnade (92) obtained several hydrogenation products by partial or total reduction of the aliphatic and aromatic double bonds in stilbestrol. At 200°C. and 5000 lb. pressure three crystalline reduction products were obtained. Two of these, m.p. 145°C. and 92–94°C., respectively, have phenolic character and are believed to represent two racemic forms of 3-(*p*-hydroxyphenyl)-4-(*p*-hydroxycyclohexyl)hexane (CII).



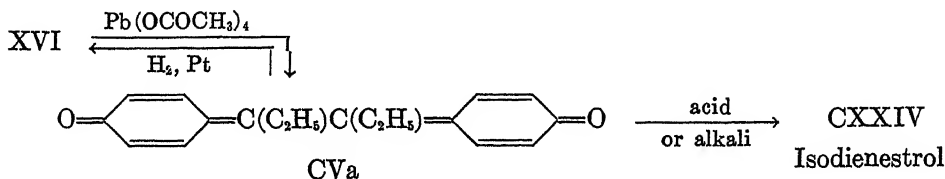
The third product, m.p. 188°C., without phenolic properties, is most likely 3,4-bis(*p*-hydroxycyclohexyl)hexane (CIII). It will be recalled that hydrogenation, under the same conditions, of the monomethyl and dimethyl ethers of stilbestrol left the aromatic rings intact, demonstrating that methylation of the phenolic hydroxyls rendered the aromatic rings more resistant to hydrogenation.

The perhydrostilbestrol, m.p. 188°C., is apparently identical with the product, m.p. 185°C., obtained earlier by Major, Christman, and Folkers (117) under similar conditions. Lane and Wallis (108) mentioned another isomeric perhydrogenation product of hexestrol, m.p. 167°C. These workers obtained from the latter, by oxidation with chromic acid, the keto alcohol (CIV) and the diketone (CV).



Closely related is α,β -diethylstilbene quinone (CVa), prepared by Euler and Adler (75a) from stilbestrol. The quinone is active at 10 micrograms, though it

was obtained neither crystalline nor analytically pure. Hydrogenation with platinum gives again stilbestrol; and with acid or alkali the quinone rearranges to isodienestrol (CXXIV), mentioned later.



Unfortunately no reports have as yet been published regarding the potency of these various cyclohexyl derivatives (table 4). However, according to Sondern (195), the products obtained by Hoehn and Ungnade (92) were inactive. If hydrogenation of one aromatic ring together with saturation of the aliphatic double bond is sufficient to abolish the activity, there is little analogy between natural and synthetic estrogens regarding the degree of saturation.

V. VARIATION OF FUNDAMENTAL STRUCTURE




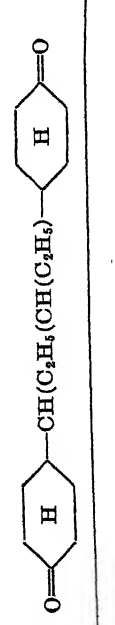
A. VARIATION OF AROMATIC RINGS

1. Acylation and alkylation of phenolic hydroxyl groups

The two phenolic hydroxyl groups present in stilbestrol and hestrol were the subject of a systematic study of the effect of conjugation on estrogenic potency. The result is a very nearly complete series of alkyl and acyl derivatives. They are generally characterized by an activity inferior to that of the parent compound. However, the minimal effective dose is but one of the factors to be considered. Another one is the ratio of oral to parenteral activity, to be discussed later together with some metabolic conversions related to it. A third factor of importance is the prolongation of estrogenic effectiveness, as measured by the duration of the estrous cycle. According to Emery *et al.* (71) the duration of stilbestrol activity depends on the site of administration, being shortest for intraperitoneal and increasingly longer for oral, intrathoracic, subcutaneous, and intramuscular injection. Sklow (200) found that, contrary to experiments made with estrone, the action of stilbestrol could not be prolonged by administration of an adsorbate on carbon powder.

The acyl derivatives of stilbestrol have been compiled in table 5, which contains information on the prolongation of effect, expressed in days of estrus duration at a given dose level. The minimal effective dose has been reported only in a few instances, but the trend may be seen of decreasing activity with increasing size of the acyl groups. The dipropionate, m.p. 107°C., appears to combine relatively high activity with extremely prolonged effect. While in the estradiol series the maximum duration was found with the higher fatty esters and the dibenzoate was found to represent the most favorable combination of characteristics, it will be noted that stilbestrol dibenzoate possesses relatively low activity and requires very large doses for prolongation. The dicarbomethoxy derivatives possess activities equivalent to those of the dipropionate. Earlier,

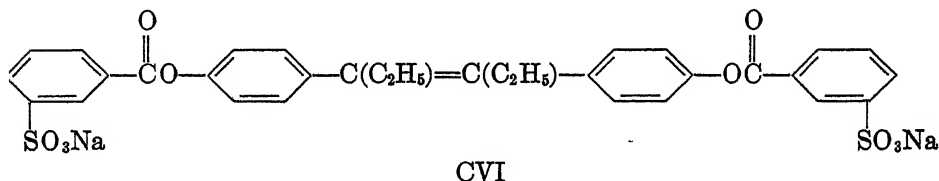
TABLE 4
Perhydrogenation products of stilbestrol and hexestrol

FORMULA	MELTING POINT °C.	NAME	REFERENCES
	185 188-188.5	3,4-Bis(<i>p</i> -hydroxycyclohexyl)hexane	(117) (92)
	(a) 145 (b) 92-94	3-(<i>p</i> -Hydroxyphenyl)-4-(<i>p</i> -hydroxycyclohexyl)hexane	(78)
	70 60	3-(<i>p</i> -Ketocyclohexyl)-4-(<i>p</i> -hydroxycyclohexyl)hexane Acetate Acid succinate	(108) (108) (108)
	80	3,4-Bis(<i>p</i> -ketocyclohexyl)hexane	(108)

in the discussion of the *cis-trans* isomerism of stilbestrol, it was mentioned that three isomeric dipropionates are known.

It should be borne in mind that some of the compounds summarized in the various tables may be the *cis*-forms rather than the *trans*-forms, as assumed. In a very few cases more than one form was isolated and it is not impossible that this one represents the biologically less active form.

Stilbestrol is soluble in water to the extent of 0.5 mg. per cent only (141); the sodium salt is soluble but gives alkaline solutions. Medick (122) esterified the phenolic hydroxyl groups with benzoic acid-3-sulfochloride. The reaction product (CVI) forms a water-soluble, neutral sodium salt, indicating that the carboxyl group took part in the esterification.



The activity has been reported equal to or even better than that of the equivalent quantity of stilbestrol. Similar esters of hexestrol and dienestrol were prepared. Among many other esters Miescher and Heer (125, 126, 127, 128, 129a, 193) described the water-soluble stilbestrol disulfate and some of its salts. Morren (135) reported that the potassium salt possesses 66 per cent of the oral activity of stilbestrol. Rabald and Boeller (151) obtained a reaction product, presumably a salt, of stilbestrol and hexamethylenetetramine. Preissecker (152) described a new derivative of uncertain composition, obtained by reacting choline with stilbestrol.

In the hexestrol series too, all previous reports agreed that esterification of the phenolic hydroxyl groups usually results in a loss of potency coupled with a prolongation of effect, as shown in table 6. Very recently it was reported by Prescott and Basden (153) that Brownlee found hexestrol dipropionate to be 1.5 to 3 times more potent than hexestrol when injected into rats. By mouth the dipropionate is less potent than hexestrol. Confirmation of these results by others will be awaited with great interest in view of the earlier report by Bretschneider *et al.* (16), who found hexestrol to be almost 3 times as active as the dipropionate. According to Foreman and Miller (83) the disuccinate of hexestrol approximates the activity of the parent substance.

Little needs to be added to the series of alkyl derivatives of stilbestrol shown in table 7 and those of hexestrol in table 6.

Alkylation of but one of the two hydroxyl groups in stilbestrol rapidly lowers the activity as the size of the alkoxy group increases. This is evidence for the view that alkyl derivatives possess little if any activity of their own but are dealkylated in the organism; alkyl derivatives are less active than esters, possibly because dealkylation under physiological conditions as well as *in vitro* offers more resistance than the hydrolysis of acyl groups. Alkyl derivatives

Acyl derivatives of stilboestrol

FORMULA $R'O\text{---}\langle\text{C}_6\text{H}_4\rangle\text{---}C(C_6H_5)=C(C_6H_5)\text{---}\langle\text{C}_6\text{H}_4\rangle\text{---}OR'$		MEETING POINT	NAME	DURATION OF ESTRUS		REFERENCES
R'	R'			Rat dose subcutaneously	Time	
		°C.		micrograms	days	
H	H	171	Stilbestrol	1 4 5 4 10 5 1	4 4 4 5 2	(57, 59) (57, 59) (57, 59) (57, 59) (98, 222)
CH ₃ CO	CH ₃ CO	124	Stilbestrol diacetate	1 1 10	0 2-3 21	(57, 58, 59) (98, 222) (57)
CH ₃ CH ₂ CO	H	92-94	Stilbestrol monopropionate			(126)
CH ₃ CH ₂ CO	CH ₃ CH ₂ CO	107	Stilbestrol dipropionate			(57, 59, 126, 129, 146, 192, 215, 220, 221)
				1.25 2.5-4.5 10	4-5 60-80 52	(221, 222) (221) (57, 59)
CH ₃ (CH ₂) ₂ CO	CH ₃ (CH ₂) ₂ CO	98-99 88	Stilbestrol di- <i>n</i> -butyrate	10	10	(129, 192) (57, 59)
(CH ₃) ₂ CHCO (CH ₃) ₂ CHCO	H (CH ₃) ₂ CHCO	109-111 101-102 86-87	Stilbestrol monoisobutyrate Stilbestrol diisobutyrate			(129, 192) (129, 192) (57, 59)
CH ₃ (CH ₂) ₃ CO	CH ₃ (CH ₂) ₃ CO	89 76.5-77.5	Stilbestrol divalerate	10	4 2	(57, 59) (129, 192)

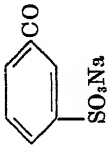
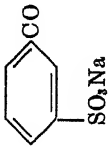
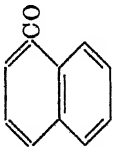
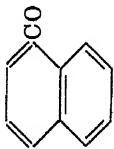
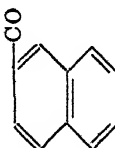
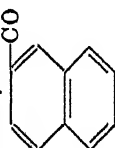
$\text{CH}_3(\text{CH}_2)_4\text{CO}$	H		Stilbestrol monocaproate			(129, 192)
$\text{CH}_3(\text{CH}_2)_4\text{CO}$	$\text{CH}_3\text{CH}_2\text{CO}$		Stilbestrol monocaproate mono-propionate			(129, 192)
$\text{CH}_3(\text{CH}_2)_4\text{CO}$	$\text{CH}_3(\text{CH}_2)_4\text{CO}$	75-76	Stilbestrol dicaproate			(129, 192)
$\text{CH}_3(\text{CH}_2)_8\text{CO}$	$\text{CH}_3(\text{CH}_2)_8\text{CO}$	67-68	Stilbestrol dicaproate			(129, 192)
$\text{CH}_3(\text{CH}_2)_{10}\text{CO}$	H		Stilbestrol monolaurate			(129, 192)
$\text{CH}_3(\text{CH}_2)_{10}\text{CO}$	$\text{CH}_3(\text{CH}_2)_{10}\text{CO}$		Stilbestrol dilaurate			(129, 192)
$\text{CH}_3(\text{CH}_2)_{13}\text{CO}$	$\text{CH}_3(\text{CH}_2)_{13}\text{CO}$	82-84 77-78	Stilbestrol dipalmitate	50 100	2 >77	(129, 192) (57, 59) (57)
$\text{CH}_3(\text{CH}_2)_{16}\text{CO}$	$\text{CH}_3(\text{CH}_2)_{16}\text{CO}$	84-86	Stilbestrol distearate			(129, 192)
$\text{C}_6\text{H}_5\text{CO}$	$\text{C}_6\text{H}_5\text{CO}$	210-211 220-222	Stilbestrol dibenzoate	10 100	0 >77	(57, 59) (57, 59) (192)
			Stilbestrol di(3-sulfobenzoate) sodium salt			(122)
$\text{C}_6\text{H}_5\text{CH}_2\text{CO}$	$\text{C}_6\text{H}_5\text{CH}_2\text{CO}$	100	Stilbestrol di(phenyl acetate)	10	4	(57, 59)
		206-207	Stilbestrol di- α -naphthoate	100	3	(59)

TABLE 5—Concluded

FORMULA $R'O-C_6H_4-C(C_6H_5)=C(C_6H_5)-C_6H_4-OR'$		MELTING POINT °C.	NAME	DURATION OF ESTRUS		REFERENCES
R'	R'			Rat dose subcuta- neously micrograms	Time days	
		252-253	Stilbestrol di-β-naphthoate	100	3	(59)
CH ₃ OCO	CH ₃ OCO	142	Stilbestrol di(methyl carbonate)	1.25	4-5	(121, 222)
C ₂ H ₅ OCO	C ₂ H ₅ OCO	118	Stilbestrol di(ethyl carbonate)	1.25	4-5	(121, 222)
(CH ₃) ₂ CHOCO	(CH ₃) ₂ CHOCO	121	Stilbestrol di(isopropyl carbon- ate)			(121)
C ₆ H ₅ OCO	C ₆ H ₅ OCO		Stilbestrol di(phenyl carbonate)	1.25	4-5	(222)
PO ₃ H ₂	PO ₃ H ₂		Stilbestrol diphosphate Calcium salt Sodium salt			(127, 128) (127, 128) (127, 128)
SO ₃ H	SO ₃ H		Stilbestrol disulfate Sodium salt Potassium salt			(127, 128) (127, 128) (135)

are stored in the organism; Dodds *et al.* (57) found that very large doses of the little active stilbestrol dimethyl ether produced almost indefinite duration of estrus in rats: more than 126 days with 1 mg. and more than 180 days with 6.6 mg. While several esters, especially the diacetate and the dipropionate of stilbestrol and hexestrol, found clinical use, to date none of their dialkyl derivatives has acquired practical importance.

Mixed ether-esters shown in table 8— for example, the monomethyl ether monoacetate of stilbestrol prepared by Ludwig (116)—appear to combine fairly high activity with prolonged effect. Stilbestrol monoacetate monotetraacetylglucoside, prepared by Miescher *et al.* (126) and by Wessely *et al.* (221), has an activity of 2.5 to 4 micrograms, slightly higher than that of the monomethyl ether monoacetate. This confirms the view that the activity of alkyl derivatives is mostly dependent on the ease of dealkylation, with the glucosidic linkage under physiological conditions more easily hydrolyzed than the methoxyl group.

2. Position isomers and homologs with additional ring substitutes

While certain other modifications of the fundamental structures of stilbestrol and hexestrol are permissible without serious loss of potency, all but a fraction of it is lost by varying the position of the substituents of the aromatic rings. From the data assembled in tables 9 and 10, it is apparent that for maximum potency both phenolic hydroxyl groups need to be in the para-position to the aliphatic part of the molecule. Linnell and Sharma (114) demonstrated that moving one of the two hydroxyls in stilbestrol from the para- to the meta-position causes a 10,000-fold drop in activity; a further drop results from moving the second hydroxyl group into the meta-position.

The relative activities of the isomers regarding the position of the two hydroxyl groups are available in three series of estrogens: those of stilbestrol, dienestrol, and the pinacol of *p*-hydroxypropiophenone. The *p,p'*-derivative is always the most active one, while the relative activities of the *m,m'*- and of the *o,o'*-isomers do not run parallel within the three series:

Approximate relative activities (within isomeric series).

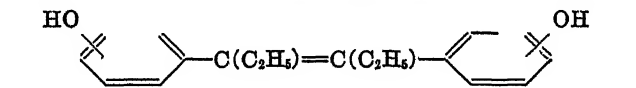
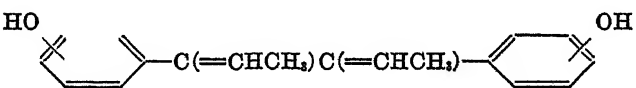
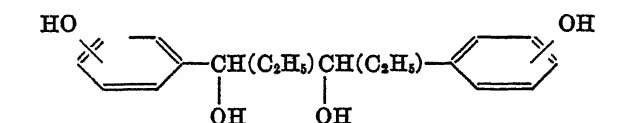
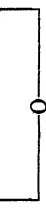
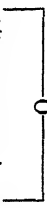
	<i>p,p'</i>	<i>m,m'</i>	<i>o,o'</i>
	1	1/30,000	1/3,000
	1	1/1,250	1/25,000
	1	1/1,000	Inactive

TABLE 6
Hexestrol, isohexestrol, and ethers and esters of hexestrol

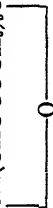


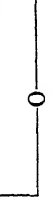
<div> <div>FORMULA</div> <div> $\text{RO}-\text{C}_6\text{H}_4-\text{CH}(\text{C}_6\text{H}_5)\text{CH}(\text{C}_6\text{H}_5)-\text{C}_6\text{H}_4-\text{OR}'$ </div> </div>		MELTING POINT	NAME	ESTROGENIC ACTIVITY IN RATS (*IN MICE) SURCUTANEOUSLY (100 PER CENT RESPONSE UNLESS INDICATED OTHERWISE)		REFERENCES
R	R'	°C.		Minimum effective dose <i>micrograms</i>	Rat units per gram	
H	H	185	Hexestrol	0.2 0.15 (75%) 2	5,000,000	(24, 56) (16) (223)
CH ₃	H	120-121	Hexestrol monomethyl ether	8 (50%)		(16, 85)
CH ₃	C ₂ H ₅ CO	119-120	Hexestrol monomethyl ether monopropionate			(92)
C ₄ H ₉	H	85-87	Hexestrol monobutyl ether	50 (50%)		(16, 144)
C ₆ H ₁₁	H		Hexestrol monoamyl ether	150		(85)
CH ₃	CH ₃	145-146	Hexestrol dimethyl ether	50 (50%)		(85, 98)
C ₆ H ₁₁	C ₆ H ₁₁		Hexestrol diamyl ether	600 (50%)		(85)
CH ₃ CO	CH ₃ CO	139	Hexestrol diacetate	1		(98)
C ₂ H ₅ CO	H	120-128	Hexestrol monopropionate	0.4 (75%)		(144)
C ₂ H ₅ CO	C ₂ H ₅ CO		Hexestrol dipropionate			(16, 83, 153, 215)

SYNTHETIC ESTROGENS

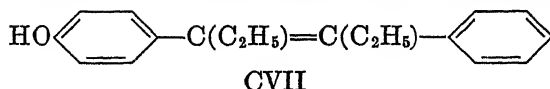
C_3H_7CO	C_3H_7CO	106-107	Hexestrol dibutyrate	4*	(83)
$CH_3(CH_2)_4CO$	$CH_3(CH_2)_4CO$	96-97	Hexestrol dicaproate	4*	(83)
$HOOC(CH_2)_2CO$	$HOOC(CH_2)_2CO$	150-153	Hexestrol disuccinate	4*	(83)
C_6H_5CO	H	123-126	Hexestrol monobenzoate		(126)
C_6H_5CO	C_6H_5CO	236-237	Hexestrol dibenzoate	4*	(83)
CH_3OCO	CH_3OCO	145-146	Hexestrol dimethylcarbonate		(27)
$CH_3OCOCH_2CH(CHOCOCCH_3)_2CH$ 	H	203-204	Hexestrol mono(tetraacetyl)glucoside		(126, 129a)
$CH_3OCOCH_2CH(CHOCOCCH_3)_2CH$ 	C_6H_5CO	156	Hexestrol mono(tetraacetyl)glucoside monobenzoate		(126, 129a)
H	H	129 130 80 80	Isohexestrol + Antipode - Antipode	500 100 1000 (40%)	(58) (223, 225) (225) (225)
C_2H_5CO	C_2H_5CO	Liquid	Isohexestrol dipropionate		(146)
CH_3OCO	CH_3OCO	95	Isohexestrol dimethyl carbonate		(27)

<div> <div>FORMULA</div> <div> $RO-\text{C}_6\text{H}_4-\text{C}(\text{CH}_3)=\text{C}(\text{CH}_3)-\text{C}_6\text{H}_4-\text{OR}'$ </div> </div>		MELTING POINT	NAME	ESTROGENIC ACTIVITY IN RATS SURTITANFOLIV (100 PER CENT RESPONSE UNLESS INDICATED OTHERWISE): MINIMUM EFFECTIVE DOSE	REFERENCE
R	R'	°C.		micrograms	
H	H	171	Stilbestrol	0.4	(56, 59)
CH ₃	H	114 117	Stilbestrol monomethyl ether	7.5 2.5 (50%)	(221) (85, 157, 171)
		118 101-102		2.5 (50%)	(116) (18)
CH ₃	CH ₃	124	Stilbestrol dimethyl ether (<i>trans</i>)	20 (50%)	(57, 58, 157)
		Liquid	Stilbestrol dimethyl ether (<i>cis</i>)		(58, 59)
C ₂ H ₅	H	99.5	Stilbestrol monoethyl ether	5 (50%)	(157)
C ₂ H ₅	C ₂ H ₅	127.5	Stilbestrol diethyl ether	50 (50%)	(157)
<i>n</i> -C ₄ H ₉	H	107	Stilbestrol mono- <i>n</i> -propyl ether	17.5 (50%)	(157)
<i>n</i> -C ₄ H ₉	<i>n</i> -C ₂ H ₅	95.6	Stilbestrol di- <i>n</i> -propyl ether	250 (50%)	(157)
<i>n</i> -C ₄ H ₉	H	97.5	Stilbestrol mono- <i>n</i> -butyl ether	20 (50%)	(157)
<i>n</i> -C ₄ H ₉	<i>n</i> -C ₄ H ₉	101.6	Stilbestrol di- <i>n</i> -butyl ether	250 (50%)	(157)
<i>n</i> -C ₆ H ₁₃	H	82	Stilbestrol mono- <i>n</i> -amyl ether	48 (50%)	(157)
<i>n</i> -C ₆ H ₁₃	<i>n</i> -C ₆ H ₁₃	64.6	Stilbestrol di- <i>n</i> -amyl ether	600 (50%)	(157)
C ₈ H ₁₇	H	72	Stilbestrol monohexyl ether	45 (50%)	(157)
C ₈ H ₁₇	C ₈ H ₁₇	74.6	Stilbestrol dihexyl ether	30,000 (50%)	(157)
C ₈ H ₁₇	H	87	Stilbestrol monoheptyl ether	45 (50%)	(157)
C ₈ H ₁₇	C ₈ H ₁₇	50.4	Stilbestrol diheptyl ether	750 (50%)	(157)
C ₈ H ₁₇	H	88.5	Stilbestrol monoöctyl ether	50 (50%)	(157)
C ₈ H ₁₇	C ₈ H ₁₇	72.2	Stilbestrol dioctyl ether	50,000 (50%)	(157)
C ₈ H ₁₇	H	76	Stilbestrol monononyl ether	50 (50%)	(157)
C ₈ H ₁₇	C ₈ H ₁₇	57	Stilbestrol dinonyl ether	5,000 (50%)	(157)
C ₈ H ₁₇	H	75	Stilbestrol monodecyl ether	84 (50%)	(157)
C ₈ H ₁₇	C ₈ H ₁₇	73	Stilbestrol didecyl ether	50,000 (50%)	(157)
C ₈ H ₁₇	H	58.5	Stilbestrol monoundecyl ether	200 (50%)	(157)

TABLE 8
Mixed ether-esters of stilbestrol

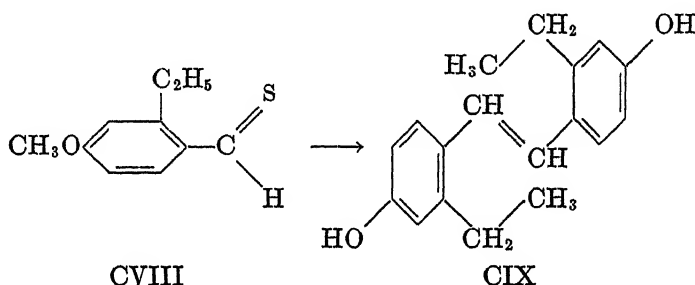
<div> <div>FORMULA</div> $\text{R}'\text{O}-\text{C}_6\text{H}_4-\text{C}(\text{C}_6\text{H}_5)=\text{C}(\text{C}_6\text{H}_5)-\text{C}_6\text{H}_4-\text{OR}'$ </div>		MELTING POINT	NAME OF ETHER-ESTER	ESTROGENIC ACTIVITY IN RATS SUBTANGOUSLY (100 PER CENT RESPONSE, UNLESS INDICATED OTHERWISE): MINIMUM EFFECTIVE DOSE	REFERENCES
R'	R'				
CH ₃	CH ₃ CO	116	Monomethyl ether monoacetate	micrograms 4.5 (50%)	(116, 170)
CH ₃	CH ₃ CH ₂ CO	76-77	Monomethyl ether monopropionate		(170)
CH ₃	CH ₃ (CH ₂) ₂ CO	65	Monomethyl ether monobutyrate		(116)
CH ₃	<i>p</i> -BrC ₆ H ₄ CO	132	Monomethyl ether mono(<i>p</i> -bromo)benzoate		(116)
CH ₃ OCOCH(CHOCOCH ₃) ₂ CH— 	C ₆ H ₅ CO		Mono(tetraacetyl-β-glucosido)monobenzoate		(126)
CH ₃ OCOCH(CHOCOCH ₃) ₂ CH— 	CH ₃ CO	152	Mono(tetraacetyl-β-glucosido)monoacetate	2.5-4.0	(126)
CH ₃ OCOCH(CHOCOCH ₃) ₂ CH— 	CH ₃ CH ₂ CO	148	Mono(tetraacetyl-β-glucosido)monopropionate		(221)
C ₂ H ₅ OCOCH(CHOCOCH ₂ H ₅) ₂ CH— 	CH ₃ CH ₂ CO	116	Mono(tetrapropionyl)glucosidomonopropionate	12-16	(221)
		119			(126)

The stilbestrol analog (CVII) containing only one hydroxyl group in the para-

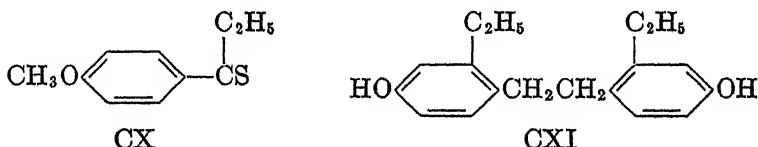


position is 1/2000 as active (59, 60) as stilbestrol; the hexestrol analog has not been assayed. Both compounds are of interest because their bactericidal activity is greater than that of dihydroxy analogs, according to Brownlee *et al.* (18) and Rubin and Wishinsky (171). The detailed discussion of their results, as well as those of Faulkner (76) and of Foley and Aycock (82), is beyond the scope of this review.

The effect of additional substituents in the rings is not consistent in the unsaturated and the saturated series. By heating 2-ethyl-4-methoxythiobenzaldehyde (CVIII) with copper powder, followed by demethylation with Grignard reagent, Linnell and Shaikmahamud (113) synthesized 2,2'-diethyl-4,4'-dihydroxystilbene (CIX).



This method was successful after various other approaches had failed, while earlier Linnell and Sharma (115) had attempted unsuccessfully to apply it to the synthesis of stilbestrol by removal of sulfur from 4-methoxythiopropiophenone (CX).



The formula of the stilbene derivative (CIX) has been written according to the authors, who interpreted its relatively low activity as evidence of the insignificance of the structural resemblance to estradiol as a requirement for high activity.

Baker (6) reported that the corresponding hexestrol analog (CXI) is inactive in doses of 100 micrograms.

Quite contrary to these results, according to Bretschneider (16) and Pallas (144), the structural isomeride (CXII) of hexestrol is practically as potent as the latter. In this case the nuclear substitution by methyl groups appears to enhance the activity, because the lower hexestrol homolog (CXIII), assayed by Dodd *et al.* (56), is less than one-half as active as hexestrol.

TABLE 9
Stilbestrol analogs with variation of aromatic substitution

FORMULA	MELTING POINT	NAME	ESTROGENIC ACTIVITY IN RATS SUBCUTANEOUSLY (* IN MICE) (100 PER CENT RESPONSE UNLESS INDICATED OTHERWISE)		REFERENCES
			Minimum effective dose	Rat units per gram	
	°C. 124	Stilbene (<i>trans</i>)	micrograms 25, 000		(55, 62)
	57-8	α,β -Diethylstilbene (<i>trans</i>)	Inactive	5, 000	(213) (171)
	147	2-Hydroxystilbene	Inactive		(64)
	189	4-Hydroxystilbene	10, 000	.	(62, 64)
	284	4,4'-Dihydroxystilbene	10, 000		(59, 62, 64)
	172	4,4'-Dihydroxy- α,β -diethylstilbene ("stilbestrol")	0.4	3, 000, 000	(56, 59)
	(a) Glass (b) Glass	3,3'-Dihydroxy- α,β -diethylstilbene Benzene-soluble form Benzene-insoluble form	3, 500* 9, 000*	(40%) (90%)	(114) (114)


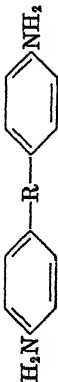




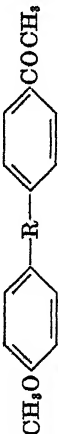
	153-154	3,4'-Dihydroxy- α,β -diethylstilbene	4,500*	(90%)	(114)
	Liquid	3-Hydroxy- α,β -diethylstilbene			(114)
	Liquid	4-Hydroxy- α,β -diethylstilbene	9,000*	(90%)	(114)
	b.p. 135-140/ 0.15 mm.	2,2'-Dihydroxy- α,β -diethylstilbene	1,000		(18, 171)
	152-153	2,2'-Dihydroxy- α,β -diethylstilbene		1,000	(56)
	231-232	3,3',4,4'-Tetrahydroxy- α,β -diethylstilbene			(13)
	227-228	3,4-Bis(<i>p</i> -hydroxyphenyl)-2,4-hexadiene ('dienestrol')	0.5	2,500,000	(54, 56, 60, 91)
	136-137	3,4-Bis(<i>o</i> -hydroxyphenyl)-2,4-hexadiene		100	(56)
	166-167	3,4-Bis(<i>m</i> -hydroxyphenyl)-2,4-hexadiene		2,000	(56)
	125 72	4,4'-Dibromo- α,β -diethylstilbene <i>trans</i> -form <i>cis</i> -form		2,500 2,500	(213) (213)

TABLE 9—Continued

FORMULA	MELTING POINT	NAME	ESTROGENIC ACTIVITY IN RATS SUBCUTANEOUSLY (* IN MICE) (100 PER CENT RESPONSE UNLESS INDICATED OTHERWISE)		REFERENCES
			Minimum effective dose	Rat units per gram	
$\text{HO}-\text{C}_6\text{H}_4-\text{C}(\text{C}_2\text{H}_5)=\text{C}(\text{C}_2\text{H}_5)-\text{C}_6\text{H}_4-\text{NH}_2$	155-156	4-Hydroxy-4'-amino- α,β -diethylstilbene	7.5 (50%)		(171)
$\text{H}_2\text{N}-\text{C}_6\text{H}_4-\text{C}(\text{C}_2\text{H}_5)=\text{C}(\text{C}_2\text{H}_5)-\text{C}_6\text{H}_4-\text{NH}_2$	132	4,4'-Diamino- α,β -diethylstilbene (<i>trans</i>) (in oily solution) Hydrochloride (in aqueous solution)		5,000	(213)
$\text{HO}-\text{C}_6\text{H}_4-\text{C}(\text{C}_2\text{H}_5)=\text{C}(\text{C}_2\text{H}_5)-\text{C}_6\text{H}_4-\text{Br}$	b.p. 170-180/ 0.1 mm.	4-Hydroxy-4'-bromo- α,β -diethylstilbene	100 (50%)	25,000	(171)
$\text{O}=\text{C}_6\text{H}_4=\text{C}(\text{C}_2\text{H}_5)\text{C}(\text{C}_2\text{H}_5)=\text{C}_6\text{H}_4=\text{O}$		α,β -Diethylstilbene quinone	10		(75a)
$\text{O}=\text{C}_6\text{H}_4=\text{C}(\text{CH}_3)\text{C}(\text{CH}_3)=\text{C}_6\text{H}_4=\text{O}$		α,β -Dimethylstilbene quinone			(75a)
$\text{Br}-\text{C}_6\text{H}_4-\text{C}(\text{CH}_3)=\text{C}(\text{CH}_3)-\text{C}_6\text{H}_4-\text{Br}$	125-126 90-91	4,4'-Dibromo- α,β -dimethylstilbene <i>trans</i> -form <i>cis</i> -form			(9, 10) (9, 10)
$\text{HO}-\text{C}_6\text{H}_3(\text{C}_2\text{H}_5)-\text{CH}=\text{CH}-\text{C}_6\text{H}_3(\text{C}_2\text{H}_5)-\text{OH}$	150	2,2'-Diethyl-4,4'-dihydroxystilbene	1,000		(113)

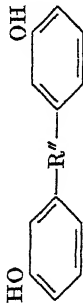
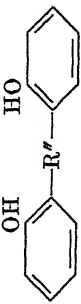
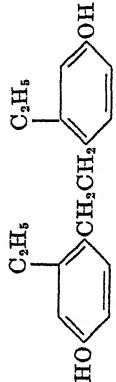
	160-161	3-Carboxy-4'-hydroxy- α,β -diethylstilbene		(111)
	167	4-Carboxy-4'-methoxy- α,β -diethylstilbene	Inactive at 1,000	(95, 171)
	80-110	3-Acetyl-4'-hydroxy- α,β -diethylstilbene		(111)
	102	4-Acetyl-4'-hydroxy- α,β -diethylstilbene	10,000	(95)
	65-67	3-(ω -Hydroxyacetyl)-4'-hydroxy- α,β -diethylstilbene	Corticosterone-like activity	(111)
	b.p. 202-206/ 0.2 mm.	4-Hydroxy-4'-acetyl- α -methyl- β -ethylstilbene	10,000	(95)
		4-Hydroxy-4'- α -hydroxyisopropyl- α -methyl- β -ethylstilbene	200	(95)
	146-148	4,4'-Dihydroxydiacetyl- α,β -diethylstilbene		(218)
	Oil	4-Methoxy-4'-hydroxy-3'-acetyl- α,β -diethylstilbene		(218)

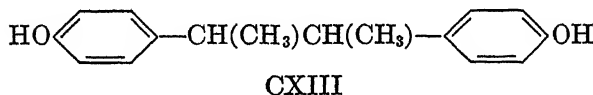
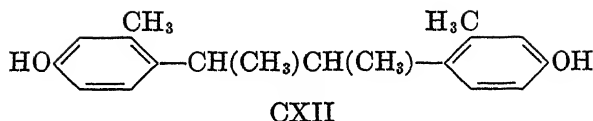
TABLE 10
Hexestrol analogs with variation of aromatic substitution

FORMULA $R = [-CH(C_2H_5)CH(C_2H_5)-]$	MELTING POINT °C.	NAME	ESTROGENIC ACTIVITY IN RATS (*IN MICE) SUBCUTANEOUSLY (100 PER CENT RESPONSE UNLESS INDICATED OTHERWISE)		REFERENCES
			Minimum effective dose <i>micrograms</i>	Rat units per gram	
	186	Hexestrol	0.2	5,000,000	(56)
	140 182-184	3,4-Bis(<i>p</i> -aminophenyl)- hexane <i>meso</i> -form <i>meso</i> -form	Inactive at 200	100	(27, 56)
	63-65	Racemate	Inactive at 200		(6)
		3,4-Bis(<i>p</i> -acetylamino- phenyl)hexane			(56)
	226-228 113-115	3,4-Bis(<i>p</i> -hydroxy- <i>m</i> -nitro- phenyl)hexane <i>d,l</i> -form <i>meso</i> -form			(199)
	139-141	3-(<i>p</i> -Anisyl)-4-(<i>p</i> -monochlo- roacetylphenyl)hexane	Progesterone-like activity		(199)
	135-136	3-(<i>p</i> -Anisyl)-4-(<i>p</i> -acetyl- phenyl)hexane	Progesterone-like activity		(18a, 218)

$\text{CH}_2\text{ClCOO}-\text{C}_6\text{H}_4-\text{R}-\text{C}_6\text{H}_4-\text{COCH}_2\text{Cl}$	135-136	3-(<i>p</i> -Monochloroacetyl-phenyl)-4-(<i>p</i> -monochloro-acetoxyphenyl)hexane	Progesterone-like activity	(18a, 218)
$\text{CH}_3\text{O}-\text{C}_6\text{H}_4-\text{R}-\text{C}_6\text{H}_4-\text{COCH}_2\text{OH}$		3-(<i>p</i> -Anisyl)-4-(<i>p</i> -glycolyl-phenyl)hexane Acetate	Corticosterone-like activity	(18a, 218)
$\text{HO}-\text{C}_6\text{H}_3(\text{OH})-\text{R}-\text{C}_6\text{H}_3(\text{OH})-\text{OH}$	231-232	3,4-(<i>m, p</i> -Dihydroxyphenyl)-hexane	Corticosterone-like activity	(218)
$\text{R}' = [-\text{CH}(\text{CH}_3)\text{CH}(\text{CH}_3)-]$				(13)
$\text{Br}-\text{C}_6\text{H}_4-\text{R}'-\text{C}_6\text{H}_4-\text{Br}$	160-161 b.p. 166-171/ 0.3 mm.	2,3-Bis(<i>p</i> -bromophenyl)-butane <i>meso</i> -form Racemate		(10)
$\text{CH}_3-\text{C}_6\text{H}_4-\text{R}'-\text{C}_6\text{H}_4-\text{OH}$	191-192 164 123-124	2,3-Bis(<i>o</i> -methyl- <i>p</i> -hydroxy-phenyl)butane Diacetate Dipropionate	0.3 (75%)	(16) (16) (16)
$\text{R}' = \left[\begin{array}{c} \text{C}_2\text{H}_5 \quad \text{C}_2\text{H}_5 \\ \quad \\ -\text{CH}-\text{CH}- \\ \quad \\ \text{OH} \quad \text{OH} \end{array} \right]$				
$\text{HO}-\text{C}_6\text{H}_4-\text{R}''-\text{C}_6\text{H}_4-\text{OH}$	204-206 94-95	3,4-Bis(<i>p</i> -hydroxyphenyl)-3,4-hexanediol Isomer	100,000	(56, 58, 59, 91)

TABLE 10—Concluded

FORMULA $R' = \left[\begin{array}{c} C_2H_5 \\ \\ -CH-CH- \\ \quad \\ OH \quad OH \end{array} \right] \begin{array}{c} C_2H_5 \\ \\ -CH- \\ \\ OH \end{array}$	MELTING POINT °C.	NAME	ESTROGENIC ACTIVITY IN RATS (*IN MICE) SUBCUTANEOUSLY (100 PER CENT RESPONSE UNLESS INDICATED OTHERWISE)		REFERENCES
			Minimum effective dose	Rat units per gram	
  	145-146	3,4-Bis(<i>m</i> -hydroxyphenyl)- 3,4-hexanediol	<i>micrograms</i>	100	(56)
	270 162	3,4-Bis-(<i>o</i> -hydroxyphenyl)- α -form β -form	Inactive Inactive		(56) (56)
	131-133	1,2-Bis(<i>m</i> -ethyl- <i>p</i> -hydroxy- phenyl)ethane	Inactive at 100		(6)



The positive effect of methyl substitution will again be encountered in the triphenylmethane series, to be discussed later. Only brief mention needs to be made of analogs containing in the para-position aromatic substituents other than hydroxyl groups. Vargha and Kovacs (213) prepared several bromo and amino derivatives of medium activity. The dibromo analog of stilbestrol (LXXXIV) is noteworthy because the *cis*- and *trans*-isomerides do not differ in potency. One might be inclined to ascribe the activity of these compounds, moderate as it may be, to physiological conversion into the dihydroxy derivatives, but this appears doubtful in view of lower activities of the corresponding compounds in the hexestrol series.

Several analogs listed in tables 9 and 10 have been prepared with the objective of modifying the estrogenic substances in a manner corresponding to the structural differences between estradiol and the steroid hormones progesterone and corticosterone. For compounds containing the hydroxyacetyl group, feeble though definite corticosterone-like activities have been reported by Linnell and Roushdi (111) and also by Brownlee and coworkers (18a, 218), who furthermore found progesterone-like activity with synthetics carrying the acetyl group characteristic for progesterone. Similar preparations were described by Jaeger and Robinson (95); their products, listed in table 9, did not exhibit progesterone-like activity but, according to the authors, this might be due to their estrogenic properties, which are of an order sufficient to inhibit progestational activity.

Though as yet most of these results are only slightly encouraging, the material available is not extensive enough to rule out the future possibility of developing synthetics sufficiently active to replace natural hormones other than estrogenic. Nevertheless, for all practical purposes estrogenic activity remains to date the only one where the steroid structure of the natural product is not essential.

B. VARIATION OF ALIPHATIC CHAIN

1. Homologs of stilbestrol and hexestrol

By replacing the two ethyl groups with other alkyl groups a large number of homologs of stilbestrol (table 11) and hexestrol (table 2) have been prepared, most of them by Dodds and his collaborators (56, 58, 59). The majority of these compounds were accessible by means of the original synthesis starting from desoxyanisoin. Incidentally, none of the many homologs have an activity equal to that of the diethyl derivatives prepared among the very first. Either loss or gain of one single methylene group causes a reduction to one-half of the potency. The same effect is obtained by keeping the molecular weight intact but

TABLE 11
Stilbestrol homologs with varied substitution on ethylene linkage

FORMULA $\text{HO}-\text{C}_6\text{H}_4-\text{C}(\text{R}')=\text{C}(\text{R}'')-\text{C}_6\text{H}_4-\text{OH}$		MELTING POINT	NAME	ESTROGENIC ACTIVITY IN RATS (*IN MICE) SUBCUTANEOUSLY (100 PER CENT RESPONSE UNLESS INDICATED OTHERWISE)		REFERENCES
R'	R''			Minimum effective dose	Rat units per gram	
H	H	°C. 284	4,4'-Dihydroxystilbene	10,000	140	(56, 59, 98)
CH ₃	H	181-182 176-177	4,4'-Dihydroxy- α -methylstilbene Diacetate	1,000 (90%)	1,000	(56) (56)
C ₂ H ₅	H	128-129 100-102	4,4'-Dihydroxy- α -ethylstilbene Dibenzoate	100 (50%)	5,000	(56, 59) (59)
<i>n</i> -C ₃ H ₇	H	91	4,4'-Dihydroxy- α - <i>n</i> -propylstilbene	200		(56)
Iso-C ₃ H ₇	H	166	4,4'-Dihydroxy- α -isopropylstilbene	500	2,000	(56)
<i>n</i> -C ₄ H ₉	H	114	4,4'-Dihydroxy- α - <i>n</i> -butylstilbene	1,000 1,000*	1,000	(37, 56) (131, 132)
Iso-C ₄ H ₉	H	128	4,4'-Dihydroxy- α -isobutylstilbene	500	2,000	(56)
<i>n</i> -C ₆ H ₁₁	H	96	4,4'-Dihydroxy- α - <i>n</i> -amylstilbene	10,000	100	(56)
C ₁₀ H ₂₁	H	b.p. 268-275/ 0.15 mm.	4,4'-Dihydroxy- α -cetylstilbene	100,000	10	(56)
		62-63	Di- β -naphthoate Dibenzoate			(56) (56)
C ₈ H ₁₇	H	99-100	4,4'-Dihydroxy- α -phenylstilbene	100	10,000	(56, 59)


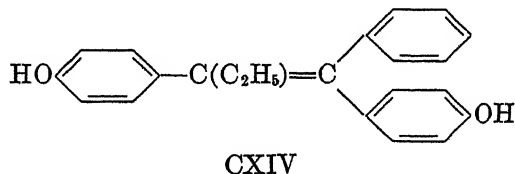
	H	136	4,4'-Dihydroxy- α -cyclohexylstilbene	2,000	500	(56)
	H	136	4,4'-Dihydroxy- α -cyclohexylstilbene	2,000	500	(56)
CH ₃	CH ₃	193-194	4,4'-Dihydroxy- α,β -dimethylstilbene	30	40,000	(56, 58, 59)
CH ₃	C ₂ H ₅	179-180 175-176	4,4'-Dihydroxy- α -methyl- β -ethylstilbene Dipropionate	1	1,000,000	(56, 59) (55) (129)
CH ₃	<i>n</i> -C ₄ H ₇	b.p. 201-202/ 0.23 mm.	4,4'-Dihydroxy- α -methyl- β - <i>n</i> -propylstilbene	0.5	1,000,000	(56)
		131-132 158	Mixture of isomers <i>trans</i> -form	1*		(131, 132) (56)
		202-204 140-141	<i>trans</i> -dibenzoate <i>cis</i> -dibenzoate			(56) (56)
CH ₃	Iso-C ₈ H ₇	158	4,4'-Dihydroxy- α -methyl- β -isopropylstilbene	0.5*		(131, 132)
C ₂ H ₅	C ₂ H ₅	171	4,4'-Dihydroxy- α,β -diethylstilbene <i>trans</i> -form = "stilbestrol"	0.4	3,000,000	(56, 58, 59)
		151	<i>cis</i> -form = ψ -stilbestrol			
		116-117	Diacetate			
		78-79	Dipropionate			
		193-194	Dibenzoate			
C ₂ H ₅	<i>n</i> -C ₄ H ₇	198-200/0.14 mm.	4,4'-Dihydroxy- α -ethyl- β - <i>n</i> -propylstilbene	10	300,000	(56, 59)
<i>n</i> -C ₂ H ₇	<i>n</i> -C ₄ H ₇	198-201/0.09 mm.	4,4'-Dihydroxy- α,β -di- <i>n</i> -propylstilbene	100	50,000	(56, 59)
Iso-C ₂ H ₇	Iso-C ₄ H ₇	202-204/0.25 mm.	4,4'-Dihydroxy- α,β -diisopropylstilbene Dipropionate	100	20,000	(56, 59) (129)

TABLE 11—*Concluded*

FORMULA $\text{HO}-\text{C}_6\text{H}_4-\text{C}(\text{R}')=\text{C}(\text{R}'')-\text{C}_6\text{H}_4-\text{OH}$		MELTING POINT	NAME	ESTROGENIC ACTIVITY IN RATS (*IN MICE) SUBCUTANEOUSLY (100 PER CENT RESPONSE UNLESS INDICATED OTHERWISE)		REFERENCES
R'	R''			Minimum effective dose	Rat units per gram	
		°C.		<i>micrograms</i>		
$n\text{-C}_4\text{H}_9$	$n\text{-C}_4\text{H}_9$	191–192/0.2 mm.	4,4'-Dihydroxy- α,β -di- <i>n</i> -butylstilbene	100 (40%)	5,000	(56, 59)
			Dibenzoate			(8)
C_2H_5	$\text{C}_{10}\text{H}_{23}$	90–91	4,4'-Dihydroxy- α -ethyl- β -cetylstilbene	3,000	500	(56)
C_6H_{11}	$\text{C}_{10}\text{H}_{23}$	98	4,4'-Dihydroxy- α -amyl- β -cetylstilbene	Inactive		(56)
		140–141	Di(3,5-dinitrobenzoate)			(56)
C_2H_5	C_6H_5	177–178	4,4'-Dihydroxy- α -ethyl- β -phenylstilbene	5	400,000	(56)
$\text{C}_6\text{H}_5\text{CH}_2$	$\text{C}_6\text{H}_5\text{CH}_2$	181–182	4,4'-Dihydroxy- α,β -dibenzylstilbene	Inactive		(56)
		160–161	(a) (b)			
CN	CN	287 217	4,4'-Dihydroxy- α,β -dicyanostilbene Diacetate	Weakly active (assayed other- wise)		(139) (139)
	$\text{O}=\text{C}(\text{CH}_3)_2$	163–164	4,4'-Dimethoxystilbenediol diacetate	Inactive		(181)
Cl	C_2H_5	b.p. 179–184/ 0.1 mm.	4,4'-Dimethoxy- α -chloro- β -ethylstilbene		1,000	(56)
Cl	Cl		4,4'-Dimethoxy- α,β -dichlorostilbene		1,000	(56)

replacing one of the two ethyl groups with a methyl group and the second with a *n*-propyl group. Moore and Volwiler (131, 132) found the methyl isopropyl homolog only slightly less active than stilbestrol. A beneficial effect of branched-chain alkyl substituents, as compared with straight-chain groups, is not generally found in these or other series. If one ethyl group is replaced by a phenyl ring, the result is a tenfold drop in activity which is, however, relatively slight in view of the sharp peak of activity for the diethyl derivative in the alkyl-substituted series. The fairly high activity (5 micrograms) of 4,4'-dihydroxy- α -ethyl- β -phenylstilbene (CXIV) is of special interest because it actually represents the



highest potency reported for the group of triphenylethylene derivatives (table 14), to be discussed separately.

Dodds *et al.* (56) demonstrated the special importance of the ethyl group; the ethyl cetyl homolog of stilbestrol is 50 times as active as the monocetyl derivatives, while the amyl cetyl homolog is inactive.

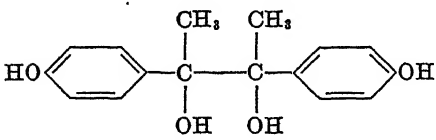
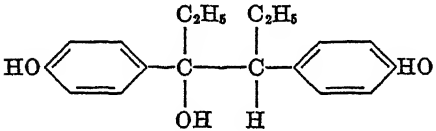
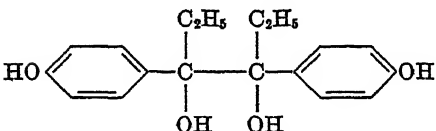
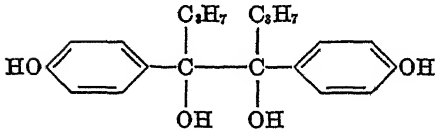
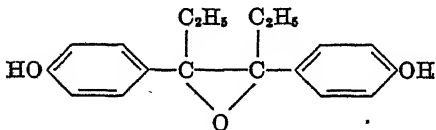
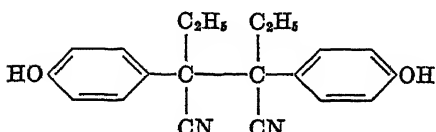
In the series of hexestrol homologs (table 2) again the question of optical isomerism needs to be considered. In the case of the dipropyl homolog Dodds *et al.* (56) described two forms and reported the crystalline isomer 200 times more active than the oily one. The same authors obtained one form of the dimethyl homolog (CXIII), m.p. 138–139°C. and fully active at 0.5 microgram, by catalytic hydrogenation and demethylation from 4,4'-dimethoxy- α,β -dimethylstilbene. Similarly Adler, Euler, and Gie (1) obtained a compound with the same melting point but found to be fully active only at the 1-mg. level. The Swedish authors obtained this isomer, believed to represent the racemate, as well as an isomer melting at 231–232°C. On account of its activity at 10 micrograms Adler *et al.* postulated that this compound represents the *meso*-form of CXIII, but the differences in activity have not yet been accounted for. The more active one of the two is about $\frac{1}{25}$ as potent as hexestrol, while about 20 times more active than the di-*n*-propyl homolog of stilbestrol. Also, the dimethyl homolog of hexestrol is no less than one-half as active as the latter, while the dimethyl homolog in the stilbestrol series is only $\frac{1}{30}$ as potent as stilbestrol. Thus the peak of activity is less sharp in the saturated than in the unsaturated series.

The activity of the racemic isohexestrol and of the products of resolution has been previously discussed.

2. Introduction of functional groups

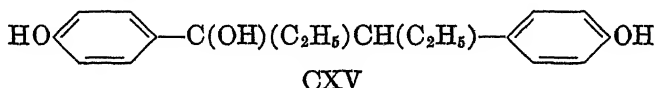
Only a few stilbestrol analogs have been described carrying functional groups on the ethylene bond. Among them are mono- and di-chloro derivatives prepared by Dodds *et al.* (56) and the dicyano derivative synthesized by Niederl and Ziering (139), all of which possess but feeble activity.

TABLE 12
Hexestrol analogs with additional functional groups

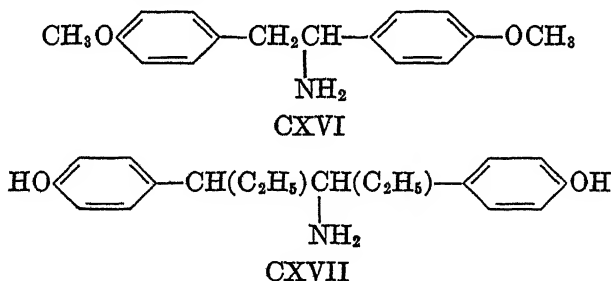
FORMULA	MELTING POINT	NAME	ESTROGENIC ACTIVITY IN RATS SUBCUTANEOUSLY (100 PER CENT RESPONSE UNLESS INDICATED OTHERWISE): MINIMUM EFFECTIVE DOSE	REFERENCES
	°C.		micrograms	
	96-97	2,3-Bis(<i>p</i> -hydroxyphenyl)-2,3-butanediol	100,000	(58, 59)
	232	3,4-Bis(<i>p</i> -hydroxyphenyl)-3-hexanol	50	(98, 222)
	153	<i>p,p'</i> -Diacetate <i>p,p'</i> -Dibenzoate	50	(98, 222) (168)
	204-206	3,4-Bis(<i>p</i> -hydroxyphenyl)-3,4-hexanediol	100	(56, 58, 59, 91)
	199-200	<i>meso</i> -form Diacetate		(58)
	94-95	Racemic form		(91)
	186-187	4,5-Bis(<i>p</i> -hydroxyphenyl)-4,5-octanediol	10,000	(58, 59)
		Diacetate		(58)
	145	4,4'-Dihydroxy- α,β -diethyl epoxystilbene	1	(98, 222)
	104	Diacetate	1	(98, 222)
	175	3,4-Bis(<i>p</i> -hydroxyphenyl)-3,4-dicyanohexane	Weakly active (tested otherwise)	(139)

As intermediates in the stilbestrol synthesis or by addition to the double bond several saturated compounds have been obtained which may be considered functional analogs of hexestrol and have been listed in table 12. Wessely *et al.* (98, 222) obtained the highly potent epoxystilbestrol (XCII) by oxidation of stilbestrol with perbenzoic acid. The configuration of the epoxy derivative has not been established.

The dimethyl ether (XLVIII) of 3,4-bis(*p*-hydroxyphenyl)-3-hexanol (CXV) and the corresponding 3,4-hexanediol (LXIII) have been mentioned earlier as intermediates of various syntheses. Considering these compounds and their homologs as functional derivatives of hexestrol we may say that the potency is reduced 100-fold owing to replacement of the first hydrogen by a hydroxyl group, and 200-fold owing to replacement of the second one.



In passing, it may be mentioned that Lettré (109) suggested a possible correlation between synthetic estrogens and mitosis poisons. He synthesized α,β -bis(*p*-anisyl)ethylamine (CXVI), which is the simplest mitosis poison related to colchicine. As proposed by Lettré, Euler *et al.* (75a) attempted, so far unsuccessfully, to prepare the diethyl derivative (CXVII), which might be formed under physiological conditions by the addition of ammonia to stilbestrol.



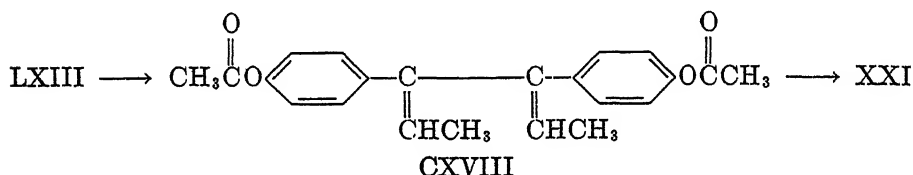
When given in large enough doses, stilbestrol itself was found to be an active mitosis poison for chicken heart fibroblasts.

3. Location and number of aliphatic double bonds

A large number of estrogens are known with the stilbene double bond shifted into a side chain or containing additional double bonds. A direct comparison between these compounds, shown in table 13, should be made with even greater reservation, on account of additional double bonds increasing the number of possible stereoisomers. Furthermore, several of these substances contain asymmetric carbon atoms, again increasing the number of possible isomers, while only in rare cases more than one isomer has been described and assayed. Among the best-studied examples regarding the effect of the configuration on the biological activity are the two forms of 3,4-bis(*p*-hydroxyphenyl)-2-hexene (XCVIII), described by Wessely *et al.* (222, 223) among the dehydration products

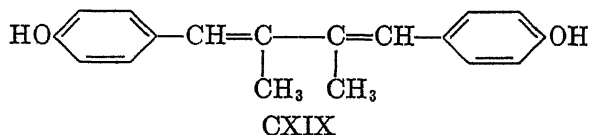
of 3,4-dianisyl-3-hexanol (XLVIII). Both stereoisomers are racemates regarding the asymmetric carbon atom. Similar to the relative potency of their structural isomers stilbestrol and ψ -stilbestrol, the higher melting form is apparently considerably more active; these results cannot be accepted without reservation because the ratio of activities has been reported as 30:1 (219) and as 400:1 (223), respectively. It is noteworthy that by means of iodine catalysis the lower melting form, presumably *cis*, is not converted in the usual manner into the higher melting one. In an equilibrium reaction both hexenes undergo isomerization to stilbestrol, involving a shift of the double bond. The speed with which the equilibrium is reached, as well as its position, is not significantly different for the two isomeric hexenes, in spite of the great difference between their potencies. Wessely (219) cited this as evidence that the hexenes are active on their own account and not by virtue of their conversion into stilbestrol. The problem of the "true" or "precursor" nature of synthetic estrogens will be discussed later from another point of view.

The most important preparation of this series is 3,4-bis(*p*-hydroxyphenyl)-2,4-hexadiene (XXI).

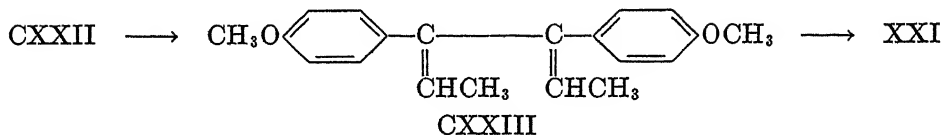
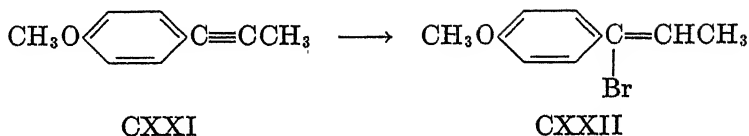
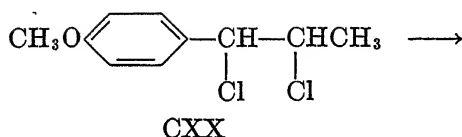


It was first prepared by Dodds, Goldberg, Lawson, and Robinson (59, 60) by way of its diacetate (CXVIII) from 3,4-bis(*p*-hydroxyphenyl)-3,4-hexanediol (LXIII) by dehydration with acetyl chloride. With the rat unit represented at 0.4 microgram this compound—also called hexadiene, hexadienestrol, or dienestrol—is one of the three most potent estrogens known.

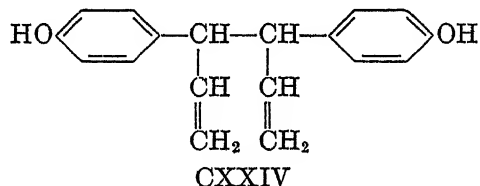
Hobday and Short (91) undertook a careful reinvestigation of a patent claim for a novel synthesis of dienestrol by Balaban and Jones (8). It could be shown by degradative oxidation of the intermediates that the product, m.p. 192°C., claimed by Balaban and Jones to be identical with dienestrol, m.p. 227–228°C., was actually 1,4-bis(*p*-hydroxyphenyl)-2,3-dimethyl-1,3-butadiene (CXIX).



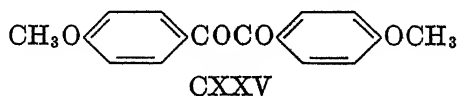
Hobday and Short developed a new synthesis of dienestrol which is analogous to that of hexestrol from anethole hydrobromide. Anethole dichloride (CXX), or the dibromide, is refluxed with pyridine and potassium methoxide to give 1-(*p*-anisyl)propyne (CXXI); the latter then adds 1 mole of hydrobromic acid resulting in 1-bromoanethole (CXXII).



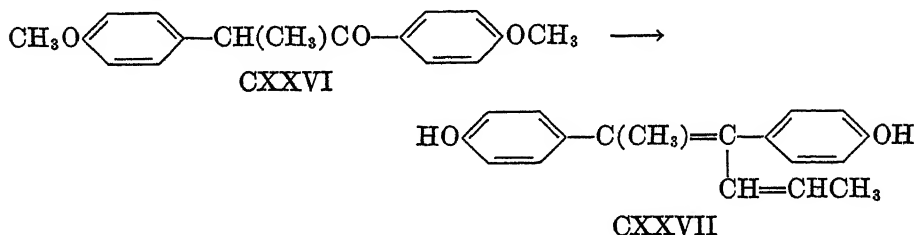
The next step comprised the successive action of magnesium and anhydrous cupric chloride to give the dimethyl ether (CXXIII), m.p. 130–131°C., of dienes-trol. Removal of the methyl groups with Grignard reagent resulted in dienes-trol, while the use of alcoholic potassium hydroxide led to a new isomeride, called isodienes-trol. Remethylation of the latter gives a liquid dimethyl ether, isomeric with CXXIII. Hydrogenation of isodienes-trol with palladized charcoal results in a mixture of 2 parts hexestrol (XX) and 1 part isohexestrol (XXII). Hobday and Short reasoned that this excluded the formula CXXIV for isodienes-trol. Barring racemization, hydrogenation would not affect the configuration in the γ, δ -position and consequently not result in a mixture of diastereomers.



Yet, racemization appears all but unlikely because the hydrogenation of dienes-trol, reported by Campbell *et al.* (25), and of isodienes-trol dimethyl ether, reported by Hobday and Short (63), gave hexestrol derivatives as the main if not exclusive products. A more convincing argument in favor of the proposed structure for isodienes-trol as one of the theoretically possible two *cis-trans* isomers of dienes-trol is the finding (91) that the dimethyl ethers of both dienes-trol and isodienes-trol are ozonized to anisil (CXXV). Euler and Adler (75a) encountered isodienes-trol as a rearrangement product of α, β -diethylstilbenequinone (CVa) and found it inactive in doses of 10 micrograms.



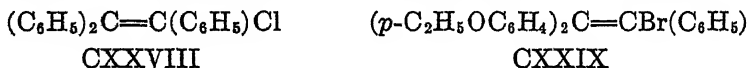
Also, in this series the activity drops off if the aliphatic side chains are either lengthened or shortened. Still very potent is 4,4'-dihydroxy- α -methyl- β -propenylstilbene (CXXVII), prepared by Moore and Volwiler (132) and subsequently by Dodds *et al.* (56). This compound was obtained by reacting methyldeoxyanisoin (CXXVI) with allylmagnesium bromide and there is no strict proof for the position of double bonds according to formula CXXVII, though the assumption was made that under the influence of alkali during demethylation the double bond shifts into the conjugated position.



From a comparison of the ethylene derivatives listed in table 11 and the dienes in table 13 it may be concluded that introduction of an additional double bond has very little effect and that, regarding the length of the aliphatic chain, the peak of activity in both series is practically the same.

VI. TRIPHENYLETHYLENE DERIVATIVES

Triphenylethylene being a phenyl-substituted stilbene, its estrogenic derivatives (table 14) may thus be considered as stilbestrol variants. Some unusual characteristics set this group apart from others. Robson and Schönberg (165) observed the notably prolonged effect of triphenylethylene, although it requires large doses. This was confirmed by Dodds, Fitzgerald, and Lawson (55), who prepared several unsubstituted di-, tri- and tetra-phenylethylenes. According to Robson, Schönberg, and Fahim (167), triphenylchloroethylene (CXXVIII) is 20 times more active than triphenylethylene and according to Segaloff (184) even 100 times. The synthesis has been described by Tadros (209a).



The enhancing effect of halogen substitution has no parallel in other series of estrogens, and the same is true for the prolonged action of a hydrocarbon not substituted with alkoxy groups. Schönberg and his collaborators (166, 167, 181) selected α,α -bis(*p*-ethoxyphenyl)- β -phenyl- β -bromoethylene (CXXIX), also known as "D.B.E.," as the most promising synthetic of this type. The method of synthesis is the same used earlier by Koelsch (102) for the corresponding dimethyl ether. The latter was also assayed by Schönberg *et al.* (181), who reported an activity about equal to that of the diethyl ether (CXXIX). Recently Davies *et al.* (39, 40) described among others α,α,β -tri(*p*-anisyl)bromoethylene (CXXX) and, in direct comparison with D.B.E. (CXXIX), found their new estrogen more effective. This last result seems to indicate that in the

TABLE 13

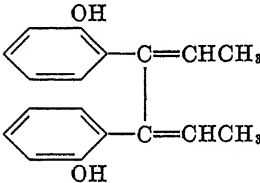
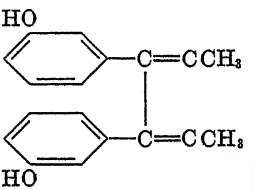
Stilbestrol analogs with variation of location or number of aliphatic double bonds

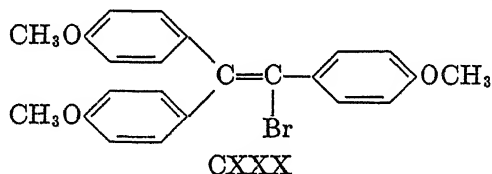
FORMULA (Disregarding <i>cis-trans</i> configuration) $R = \text{---} \text{C}_6\text{H}_4 \text{---} \text{OH}$	MELTING POINT	NAME	ESTROGENIC ACTIVITY IN RATS (* IN MICE) SUBCUTANEOUSLY (100 PER CENT RESPONSE UNLESS INDICATED OTHERWISE)		REFERENCES
			Minimum effective dose	Rat units per gram	
RCC_2H_5 RCC_2H_5	°C. 172	4,4'-Dihydroxy- α,β-diethyl- stilbene (“stilbes- trol”)	0.4 <i>micrograms</i>	3,000,000	(56, 59)
RCHC_2H_5 $\text{RCH}=\text{CHCH}_3$	(a) 143.5 (b) 153	3,4-Bis(<i>p</i> -hy- droxyphen- yl)-2-hexene Isomer	300 10 2.5		(219,222) 223) (219) (222, 223)
	(a) Liquid (b) 74	Diacetate Isomer			(223) (223)
	(a) 126 (b) 184	Dibenzoate Isomer			(223) (223)
RCCH_3 $\text{RCCH}=\text{CHCH}_3$	162 158-159	4,4'-Dihydroxy- α-methyl-β- propenylstil- bene	2 1	500,000	(56) (131, 132)
RCC_2H_5 $\text{RCCH}=\text{CHCH}_3$	b.p. 208- 211/0.17 mm.	4,4'-Dihydroxy- α-ethyl-β- propenylstil- bene	5	400,000	(56, 59)
$\text{RCCH}=\text{CHCH}_3$ $\text{RCCH}=\text{CHCH}_3$	b.p. 220- 226/0.4 mm. 164	4,4'-Dihydroxy- α,β-dipro- penylstilbene Dibenzoate	100	20,000	(56, 59) (59)
$\text{RCHCH}_2\text{CH}=\text{CH}_2$ $\text{RCHCH}_2\text{CH}=\text{CH}_2$	Glass	4,4'-Dihydroxy- α,β-diallyl- stilbene			(37)
$\text{RC}=\text{CH}_2$ $\text{RC}=\text{CH}_2$		2,3-Bis(<i>p</i> -hy- droxyphen- yl)-1,3-bu- tadiene	Inactive		(60)

TABLE 13—Continued

FORMULA (Disregarding <i>cis-trans</i> configuration) $R = \text{—}\langle \text{C}_6\text{H}_4 \rangle\text{—OH}$	MELTING POINT	NAME	ESTROGENIC ACTIVITY IN RATS (* IN MICE) SUBCUTANEOUSLY (100 PER CENT RESPONSE UNLESS INDICATED OTHERWISE)		REFERENCES
			Minimum effective dose	Rat units per gram	
	°C.		micrograms		
$\begin{array}{c} \text{RC}=\text{CHCH}_3 \\ \\ \text{RC}=\text{CHCH}_3 \end{array}$	227–228	3,4-Bis(<i>p</i> -hydroxyphenyl)-2,4-hexadiene (“dienestrol”)	0.5	1,000,000	(59, 60)
	142	Monomethyl ether			(91)
	224	Dibenzoate			(91)
	119–120	Diacetate			(59, 60)
	96	Dipropionate			(59)
	189	Isodienestrol	Inactive		(75a, 91)
$\begin{array}{c} \text{RC}=\text{CHC}_2\text{H}_5 \\ \\ \text{RC}=\text{CHC}_2\text{H}_5 \end{array}$		4,5-Bis(<i>p</i> -hydroxyphenyl)-3,5-octadiene	10		(59, 60)
$\begin{array}{c} \text{RCHCH}=\text{C}(\text{CH}_3)_2 \\ \\ \text{RCHCH}=\text{C}(\text{CH}_3)_2 \end{array}$		4,5-Bis(<i>p</i> -hydroxyphenyl)-1,8-dimethyl-2,7-octadiene	100		(59)
$\begin{array}{c} \text{RCH}=\text{CCH}_3 \\ \\ \text{RCH}=\text{CCH}_3 \end{array}$	192	1,4-Bis(<i>p</i> -hydroxyphenyl)-2,3-dimethyl-1,3-butadiene			(8, 91)
$\begin{array}{c} \text{RCH}=\text{CCH}_3 \\ \\ \text{RCHC}_2\text{H}_5 \end{array}$	Gum	1,3-Bis(<i>p</i> -hydroxyphenyl)-2-methyl-1-pentene (“dianol”)	100		(23)
$\begin{array}{c} \text{RC}=\text{CH}-\langle \text{C}_6\text{H}_5 \rangle \\ \\ \text{RC}=\text{CH}-\langle \text{C}_6\text{H}_5 \rangle \end{array}$		2,3-Bis(<i>p</i> -hydroxyphenyl)-1,4-diphenyl-1,3-butadiene	Nil		(60)
		Diacetate			(60)

TABLE 13—Concluded

FORMULA (Disregarding <i>cis-trans</i> configuration) $R = \text{---}\text{C}_6\text{H}_4\text{---OH}$	MELTING POINT	NAME	ESTROGENIC ACTIVITY IN RATS (*IN MICE) SUBCUTANEOUSLY (100 PER CENT RESPONSE UNLESS INDICATED OTHERWISE)		REFERENCES
			Minimum effective dose	Rat units per gram	
	°C. 136-137	3,4-Bis(o-hydroxyphenyl)-2,4-hexadiene	micrograms	100	(56)
	166-167	3,4-Bis(m-hydroxyphenyl)-2,4-hexadiene		3,000	(56)



triphenylethylene series fundamentally the same holds true as in the other series of related stilbene derivatives, i.e., maximum activity requires phenolic substitution of two rings attached to two different ethylene carbon atoms. This is not surprising in view of the finding of Dodds *et al.* (56), mentioned before, that 4,4'-dihydroxy- α -ethyl- β -phenylstilbene (CXIV) is not less than $\frac{1}{12}$ as active as stilbestrol and thus the most active triphenylethylene derivative reported to date. On the basis of results originating from various laboratories and listed in table 14 it appears that the halogenated triphenylethylene derivatives carrying alkoxy groups are only about $\frac{1}{4}$ as active (20 micrograms) as compound CXIV, more closely related to stilbestrol. It is true that these substances possess an extremely prolonged activity subcutaneously and orally, a characteristic not shared by the ethers and esters of the stilbestrol and other series. A comparison is difficult because most of the triphenylethylene derivatives have been assayed by a special procedure devised by Robson (162). It is certainly unfortunate that no comparative data have been published regarding the activity of the dealkylation products which may, however, not be sufficiently stable, nor those of the corresponding esters.

TABLE 14
Triphenylethylene derivatives

FORMULA	NAME	ESTROGENIC ACTIVITY IN RATS (*MICE) SUBCUTANEOUSLY (100 PER CENT RESPONSE UNLESS INDICATED OTHERWISE)		DURATION OF ESTRUS		REFERENCES
		Minimum effective dose	Rat units per gram	Days total	Days until action halved	
$\text{HO} \begin{array}{c} \\ \text{C}_6\text{H}_4 \end{array} \text{C}(\text{C}_2\text{H}_5)=\text{C}(\text{C}_2\text{H}_5)-\begin{array}{c} \text{C}_6\text{H}_4 \\ \\ \text{OH} \end{array}$	Stilbestrol	micrograms 0.1* 50* 500*			3 9 16	(166) (166) (166)
$\begin{array}{c} \text{C}_6\text{H}_5 \\ \\ \text{C}=\text{CH}_2 \\ \\ \text{C}_6\text{H}_5 \end{array}$	α,α -Diphenylethylene	Nil				(55)
$\begin{array}{c} \text{C}_6\text{H}_5 \\ \\ \text{C}=\text{C} \\ \quad \\ \text{C}_6\text{H}_5 \quad \text{C}_6\text{H}_5 \end{array}$	Triphenylethylene	1,000* 10,000* 10,000*		>110	105	(165) (166) (165, 167)
$\begin{array}{c} \text{C}_6\text{H}_5 \\ \\ \text{C}=\text{C} \\ \quad \\ \text{C}_6\text{H}_5 \quad \text{C}_6\text{H}_5 \end{array}$	Tetraphenylethylene	Inactive				(166)


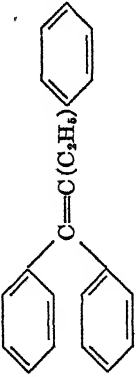
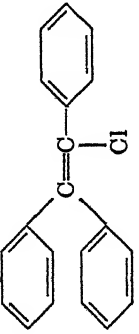
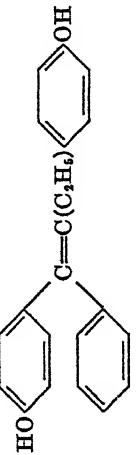
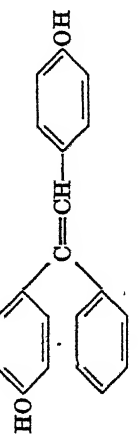
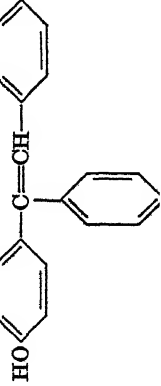
	α,β -Di(1-naphthyl)-ethylene	Inactive			(166)
	α -Ethyl- β -phenylstilbene		2,500		(56)
	α,α,β -Triphenyl- β -chloroethylene	500*		>130	(165, 167, 209a)
	4,4'-Dihydroxy- α -ethyl- β -phenylstilbene	20 per 20-g. rat	400,000		(56)
	4,4'-Dihydroxyphenylstilbene	100	10,000		(56, 59)
	α -(<i>p</i> -Hydroxyphenyl)-stilbene		4,000		(56)

TABLE 14—Continued

FORMULA	NAME	ESTROGENIC ACTIVITY IN RATS (*IN MICE) SUBSTANTIOUSLY (100 PER CENT RESPONSE UNLESS INDICATED OTHERWISE)		DURATION OF ESTRUS		REFERENCES
		Minimum effective dose	Rat units per gram	Days total	Days until action halved	
	α -(<i>p</i> -Hydroxyphenyl)- β -ethylstilbene	micrograms	30,000			(50)
	α, α -Di(<i>p</i> -anisyl)- β -phenyl- β -bromo-ethylene	20* 100*			9 68	(40, 181) (181)
	α, α -Bis(<i>p</i> -ethoxyphenyl)- β -phenyl- β -bromo-ethylene ('D.B.E.')	20* 100*			4 93	(166) (166)
	α, α, β -Triphenyl- β -cyano-ethylene	1,000 per 20-g. rat				(5)

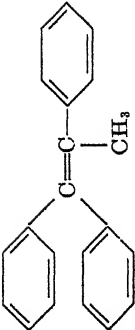
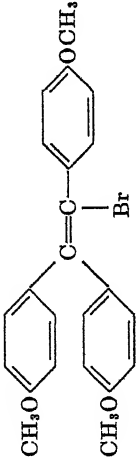
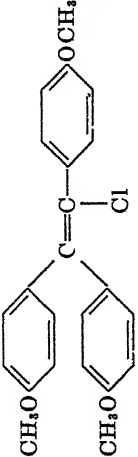
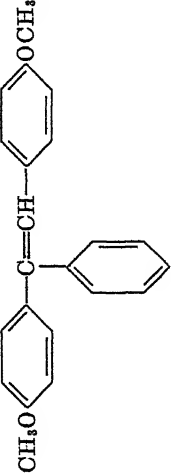
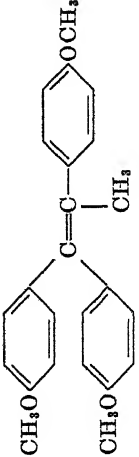
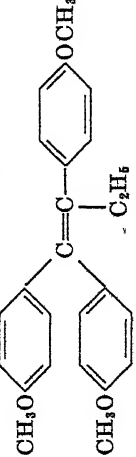
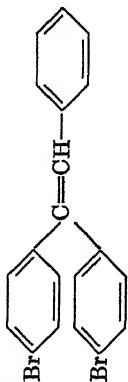
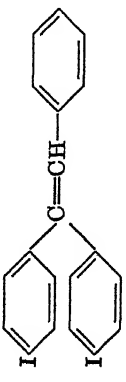
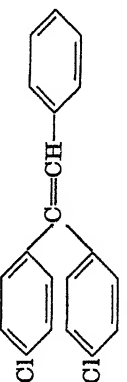
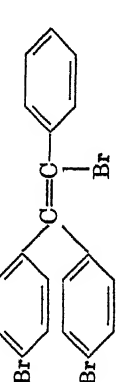
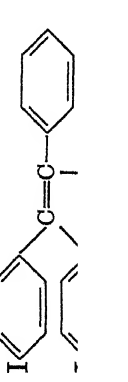
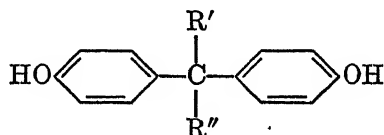
	α, α, β -Triphenyl- β -methyleneethylene				(5)
	α, α, β -Tri(<i>p</i> -anisyl)- β -bromoethylene	20*		28	(40)
	α, α, β -Tri(<i>p</i> -anisyl)- β -chloroethylene	1,000		53	(210c)
	α, β -Di(<i>p</i> -anisyl)- α -phenylethylene <i>cis-trans</i> isomers (a) (b)	200* 200*	(25%) (10%)	2	(39) (39)
	α, α, β -Tri(<i>p</i> -anisyl)- β -methyleneethylene	10*	(45%)	6	(39)
	α, α, β -Tri(<i>p</i> -anisyl)- β -ethylethylene	10* 50*	(20%) (90%)	2 19	(39) (39)

TABLE 14—*Concluded*

FORMULA	NAME	ESTROGENIC ACTIVITY IN RATS (*IN MICE) SUBCUTANEOUSLY (100 PER CENT RESPONSE UNLESS INDICATED OTHERWISE)		DURATION OF ESTRUS		REFERENCES
		Minimum effective dose	Rat units per gram	Days total	Days until action halved	
	α, α' -Bis(<i>p</i> -bromophenyl)- β -phenylethylene	5,000 inactive				(181)
	α, α' -Bis(<i>p</i> -iodophenyl)- β -phenylethylene	5,000 inactive				(181)
	α, α' -Bis(<i>p</i> -chlorophenyl)- β -phenylethylene	5,000 inactive				(181)
	α, α' -Bis(<i>p</i> -bromophenyl)- β -phenyl- β -bromo-ethylene	5,000 inactive				(181)
	α, α' -Bis(<i>p</i> -iodophenyl)- β -phenyl- β -bromo-ethylene	5,000 weakly active				(181)

[VII. DIPHENYLMETHANE DERIVATIVES

Prior to the development of stilbestrol and hexestrol Dodds and Lawson (61) found 4,4'-dihydroxydiphenylmethane (CXXXI) among the first simpler syn-



CXXXI: $R' = R'' = H$

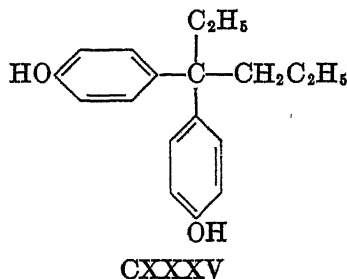
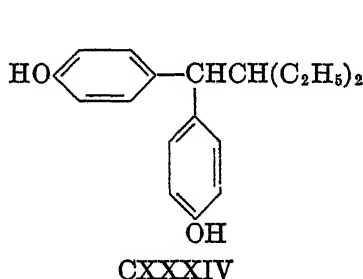
CXXXII: $R' = H$; $R'' = \text{alkyl}$

CXXXIII: $R' = \text{alkyl}$; $R'' = \text{alkyl}$

thetic estrogens not containing the phenanthrene or dibenzanthracene skeleton. These authors (64) later systematically substituted the parent compound with alkyl groups, but none of the compounds tested was active at a dose level lower than 100 mg. Thereafter the interest centered on the diphenylethane series, but more recently Campbell (22) undertook a systematic reinvestigation of the diphenylmethane series and included for assaying some compounds prepared earlier by others (44, 67, 89, 180, 231, 232). The series was finally supplemented by Reid and Wilson (158).

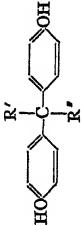
None of the compounds summarized in table 15 matches the potency of analogs in the stilbene or dihydrostilbene series. Nevertheless the study is of interest for two reasons. Firstly, it demonstrates the success of the systematic substitution of a given structure by various alkyl groups, resulting in a 500-fold increase of potency, and secondly, the series includes a number of structural isomers of hexestrol which offer an opportunity not available in other series for a study of the effect of the structural variation on the potency.

In the series of monosubstituted compounds (CXXXII) the activities are generally low. The peak (5 to 10 mg. dose level) is reached with $R = \text{diethylmethyl}$. This compound (CXXXIV) is an isomer of hexestrol (XX), the difference being the transposition of an ethyl group and one aromatic group, causing a 50,000-fold drop in activity.



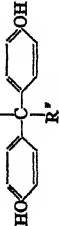
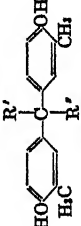
In the series of α, α -dialkyl-substituted diphenylmethanes (CXXXIII) a 5 to 10 times higher potency was found, with the peak for $R' = \text{ethyl}$ and $R'' = n\text{-propyl}$ (CXXXV), again an isomer of hexestrol with transposition of one aromatic ring and one hydrogen atom.

TABLE 15
Diphenylmethane derivatives

FORMULA 		MELTING POINT	NAME	ESTROGENIC ACTIVITY IN RATS SUBCUTANEOUSLY (100 PER CENT RESPONSE UNLESS INDICATED OTHERWISE)		REFERENCES
R'	R''			Minimum effective dose	Rat units per gram	
H	H	°C.	Bis(4-hydroxyphenyl)methane	100,000	20	(61, 192) (21)
H	CH ₃	122	1,1-Bis(4-hydroxyphenyl)ethane		20	(21, 89)
CH ₃	CH ₃	155	2,2-Bis(4-hydroxyphenyl)propane	100,000	36 28	(61, 64) (21, 231) (158)
H	C ₂ H ₅	129	1,1-Bis(4-hydroxyphenyl)propane	100,000	16	(61) (21, 89)
CH ₃	C ₂ H ₅	124	2,2-Bis(4-hydroxyphenyl)butane	100,000	124 100	(61, 64, 67, 120) (158) (21)
H	<i>n</i> -C ₃ H ₇	137	1,1-Bis(4-hydroxyphenyl)butane		40	(21, 89)
H	Iso-C ₃ H ₇	152	1,1-Bis(4-hydroxyphenyl)-2-methyl- propane		36	(21)
CH ₃	<i>n</i> -C ₃ H ₇	149	2,2-Bis(4-hydroxyphenyl)pentane	100,000	200	(61, 64, 67, 120) (21, 158)

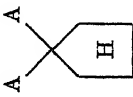
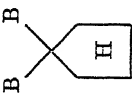
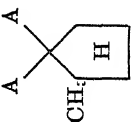
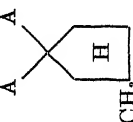
CH ₃	Iso-C ₃ H ₇	194	2, 2-Bis(4-hydroxyphenyl)-3-methyl-butane		50	(158)
C ₂ H ₅	C ₃ H ₇	204	3, 3-Bis(4-hydroxyphenyl)pentane	100, 000	200	(44, 61, 64) (158)
H	Iso-C ₄ H ₉	145	1, 1-Bis(4-hydroxyphenyl)-3-methyl-butane		100	(21)
CH ₃	n-C ₄ H ₉	Liquid	2, 2-Bis(4-hydroxyphenyl)hexane		40	(21, 158)
CH ₃	Iso-C ₄ H ₉	153	2, 2-Bis(4-hydroxyphenyl)-4-methyl-pentane		250 200	(21, 120) (158)
C ₂ H ₅	n-C ₅ H ₁₁	155	3, 3-Bis(4-hydroxyphenyl)hexane		1, 000	(21)
H	(C ₂ H ₅) ₂ CH	168	1, 1-Bis(4-hydroxyphenyl)-2-methyl-butane	7, 500	140	(21)
H	C ₆ H ₁₃	120	1, 1-Bis(4-hydroxyphenyl)heptane	100, 000	40	(61, 64) (21)
n-C ₆ H ₁₃	n-C ₇ H ₁₅	154	4, 4-Bis(4-hydroxyphenyl)heptane		500 2, 000	(21) (158)
CH ₃	C ₈ H ₁₇	101	2, 2-Bis(4-hydroxyphenyl)heptane		35	(158)
CH ₃	C ₈ H ₁₇	88	2, 2-Bis(4-hydroxyphenyl)octane		50	(158)
n-C ₈ H ₁₇	n-C ₈ H ₁₇	150	4, 4-Bis(4-hydroxyphenyl)octane		200	(21)
H	(n-C ₈ H ₁₇) ₂ CH	128	1, 1-Bis(4-hydroxyphenyl)-2-n-propylpentane		90	(21)

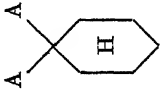
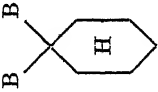
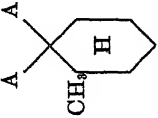
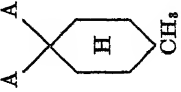
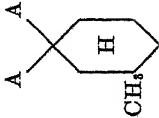
TABLE 15—Continued

FORMULA 		MELTING POINT	NAME	ESTROGENIC ACTIVITY IN RATS SUBCUTANEOUSLY (100 PER CENT RESPONSE UNLESS INDICATED OTHERWISE)		REFERENCES
R'	R''			Minimum effective dose	Rat units per gram	
$n\text{-C}_4\text{H}_9$	$n\text{-C}_4\text{H}_9$	170.5	5,5-Bis(4-hydroxyphenyl)nonane	100,000	20	(21, 158)
C_6H_{11}	C_6H_{11}	148.5	6,6-Bis(4-hydroxyphenyl)undecane		10	(158)
H	C_6H_5	161	Bis(4-hydroxyphenyl)phenylmethane			(64)
CH_3	C_2H_5	188	1,1-Bis(4-hydroxyphenyl)phenyl-ethane	100,000 (60%) Inactive		(64) (158)
CH_3	$p\text{-CH}_3\text{OC}_2\text{H}_5$	245	1,1-Bis(4-hydroxyphenyl)- <i>p</i> -anisyl-ethane		55	(158)
$p\text{-HOC}_6\text{H}_4$	$p\text{-HOC}_6\text{H}_4$	282	Tetra(4-hydroxyphenyl)methane	Inactive		(64)
$\text{C}_6\text{H}_5\text{CH}_3$	$\text{C}_6\text{H}_5\text{CH}_3$	193	Bis(4-hydroxyphenyl)dibenzyl-methane	Inactive		(158, 232)
						
R'	R''					
H	C_6H_5	94	1,1-Bis(3-methyl-4-hydroxyphenyl)-propane		8	(21, 89)

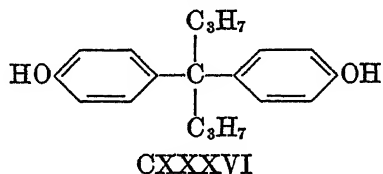
CH ₃	CH ₃	137-139	2, 2-Bis (3-methyl-4-hydroxyphenyl) - propane	100, 000	40	(21) (64)
CH ₃	C ₂ H ₅	145-147	2, 2-Bis (3-methyl-4-hydroxyphenyl) - butane	100, 000	40	(21, 67, 120) (64)
C ₃ H ₇	C ₃ H ₇	120	3, 3-Bis (3-methyl-4-hydroxyphenyl) - pentane		1, 000	(21)
CH ₃	n-C ₄ H ₉	128	2, 2-Bis (3-methyl-4-hydroxyphenyl) - pentane		250	(21)
H	Iso-C ₄ H ₉	124	1, 1-Bis (3-methyl-4-hydroxyphenyl) - 2-methylpropane		28	(21)
CH ₃	n-C ₄ H ₉	104-105	2, 2-Bis (3-methyl-4-hydroxyphenyl) - hexane		40	(21)
CH ₃	Iso-C ₄ H ₉	128	2, 2-Bis (3-methyl-4-hydroxyphenyl) - 4-methylpentane		180	(21, 120)
C ₃ H ₇	n-C ₅ H ₁₁	90	3, 3-Bis (3-methyl-4-hydroxyphenyl) - hexane		4, 000	(21)
n-C ₄ H ₉	n-C ₅ H ₁₁	173	4, 4-Bis (3-methyl-4-hydroxyphenyl) - heptane		5, 000	(21)
n-C ₄ H ₉	n-C ₆ H ₁₃	140	4, 4-Bis (3-methyl-4-hydroxyphenyl) - octane		1, 000	(21)
n-C ₄ H ₉	n-C ₇ H ₁₅	128	5, 5-Bis (3-methyl-4-hydroxyphenyl) - nonane		200	(21)

TABLE 15—*Concluded*

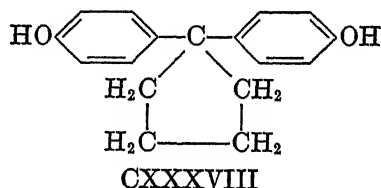
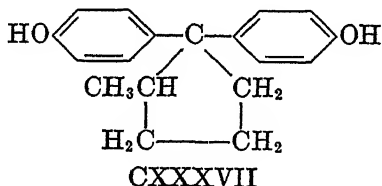
FORMULA $A = \left[\text{C}_6\text{H}_4\text{OH} \right]$ $B = \left[\text{C}_6\text{H}_4\text{CH}_3 \right]$	MELTING POINT	NAME	ESTROGENIC ACTIVITY IN RATS SUBCUTANEOUSLY (100 PER CENT RESPONSE UNLESS INDICATED OTHERWISE)		REFERENCES
			Minimum effective dose	Rat units per gram	
	°C. 156	1,1-Bis(4-hydroxyphenyl)cyclopentane	<i>micrograms</i>	15	(21, 120) (158)
				66	
	162	1,1-Bis(3-methyl-4-hydroxyphenyl)-cyclopentane	36		(21)
	161	1,1-Bis(4-hydroxyphenyl)-2-methyl-cyclopentane	100		(21)
	171	1,1-Bis(4-hydroxyphenyl)-3-methyl-cyclopentane	40		(21)

	184	1,1-Bis(4-hydroxyphenyl)cyclohexane	100,000	40	(21, 64, 120)
	191-192	1,1-Bis(3-methyl-4-hydroxyphenyl)-cyclohexane	100,000	40	(21, 64)
	235	1,1-Bis(4-hydroxyphenyl)-2-methyl-cyclohexane		25	(21)
	179	1,1-Bis(4-hydroxyphenyl)-4-methyl-cyclohexane		200	(120, 158)
		1,1-Bis(4-hydroxyphenyl)-3-methyl-cyclohexane			(120)

In both the mono- and the di-substituted series a comparison was made with homologs carrying methyl groups in the ortho-position to the two phenolic hydroxyl groups. The detrimental effect of this substitution had been reported earlier (64) for compounds of the type of CXXXI and was now confirmed by Campbell (22) in the monosubstituted series (CXXXII). Quite unexpectedly, a strong enhancing effect was found in series CXXXIII, resulting in a four- to five-fold increase over the most active homologs without nuclear methyl groups. The peak lies here with the di-*n*-propyl derivative (CXXXVI), which is not an isomer of hexestrol; its potency at the 200-microgram level represents the highest one yet found in the diphenylmethane series.



Campbell remarked that the peak for the dipropyl-substituted compound (CXXXVI) recalls the maximum potency found for the equally substituted compound (III) in the 9,10-dialkylanthraquinol series. This author also included structures where the central carbon atom is part of a ring system. The activities were 50- to 200-fold lower than in the preceding series. Within the cyclic series the highest activity lies with the α -methylcyclopentane derivative (CXXXVII), which is six to seven times as active as the lower homolog (CXXXVIII).

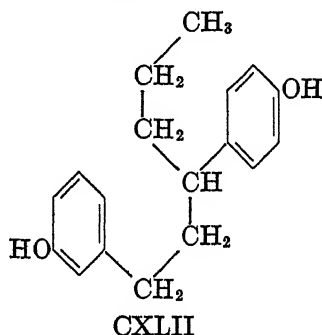
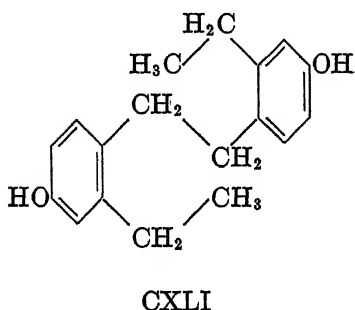
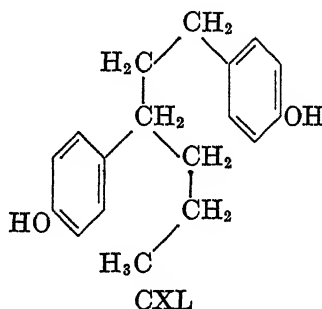
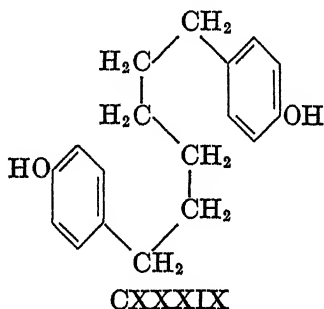


On the other hand, ortho-methylation of the cyclohexane analog of CXXXVIII, prepared by Dodds and Lawson (61), lowered the activity.

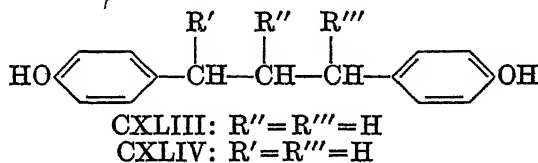
VIII. DIPHENYLPROPANE DERIVATIVES

Numerous estrogenic compounds have been investigated with longer and branched alkane chains between the two hydroxyphenyl rings (table 16). Some of these have been reported by Dodds and coworkers (25,64) for the purpose of identifying the highly active estrogen among the demethylation products of anethole. Baker (6) endeavored to obtain substances still conforming to the estradiol pattern but with its hydronaphthalene skeleton ruptured at places other than in hexestrol. Among these compounds, written according to Baker, are 1,6-bis(*p*-hydroxyphenyl)hexane (CXXXIX), 1,3-bis(*p*-hydroxyphenyl)-

hexane (CXL), and 1,2-bis(2-ethyl-4-hydroxyphenyl)ethane (CXLI, identical with CXI); all of these are practically inactive.

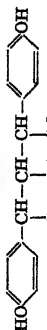


Blanchard, Stuart, and Tallman (12, 206, 207) at first followed similar lines and synthesized 1-(*m*-hydroxyphenyl)-3-(*p*-hydroxyphenyl)hexane (CXLII), which is moderately active. These authors then disregarded the hypothetical structural resemblance and prepared a series of di(*p*-hydroxyphenyl)propane derivatives and concluded, on the basis of their results, that this resemblance is coincidental. In the series of monosubstituted di(*p*-hydroxyphenyl)propane derivatives (CXLIII) the highest activity was found for $R = C_3H_7$, with the rat



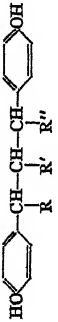
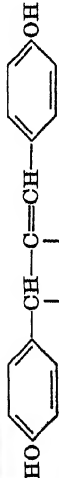

unit at 5 to 10 mg. When the substituent was attached to the central methylene group (CXLIV), the optimum activity for $R'' = C_2H_5$ was found to be of the same order as that of the unsymmetrically substituted compound (CXLIII). A decisive improvement of activity was obtained when all three aliphatic carbon atoms carried alkyl substituents. Here the optimal rat unit of 3 micrograms was found for the methyl-ethyl-ethyl substitution, resulting in 2,4-bis(*p*-hydroxyphenyl)-3-ethylhexane (CXLIX). This substance was prepared by way of the condensation product (CXLV) of anisaldehyde with *p*-methoxypropionphe-

TABLE 16
Diphenylpropane derivatives and higher homologs

FORMULA 			MELTING POINT	NAME	ESTROGENIC ACTIVITY IN RATS SUBCUTANEOUSLY (100 PER CENT RE- SPONSE UNLESS INDICATED OTHERWISE): MINIMUM EFFECTIVE DOSE	REFERENCES
R	R'	R''				
H	H	H	104-105 °C.	1, 3-Bis(<i>p</i> -hydroxyphenyl)propane	100, 000 10, 000 <i>micrograms</i>	(64, 105) (207)
CH ₃	H	H	Resin	1, 3-Bis(<i>p</i> -hydroxyphenyl)butane	<10, 000	(207)
C ₂ H ₅	H	H	99-100	1, 3-Bis(<i>p</i> -hydroxyphenyl)pentane	10, 000 Inactive at 100	(207) (6)
<i>n</i> -C ₃ H ₇	H	H	101	1, 3-Bis(<i>p</i> -hydroxyphenyl)hexane	2, 000 (70%)	(12, 207)
Iso-C ₄ H ₇	H	H	b.p. 178-179	2-Methyl-3, 5-bis(<i>p</i> -hydroxy- phenyl)pentane	<10, 000	(207)
<i>n</i> -C ₄ H ₉	H	H	Resin	5, 7-Bis(<i>p</i> -hydroxyphenyl)heptane	<10, 000	(207)
<i>n</i> -C ₆ H ₁₁	H	H	Resin	6, 8-Bis(<i>p</i> -hydroxyphenyl)octane	<10, 000	(207)
C ₆ H ₅	H	H	105-106	1-Phenyl-1, 3-bis(<i>p</i> -hydroxy- phenyl)propane	<10, 000	(207)
C ₆ H ₅ CH ₂	H	H	108-110	1-Phenyl-2, 4-bis(<i>p</i> -hydroxy- phenyl)butane	<10, 000	(207)
CH ₃ OC ₂ H ₅	H	H	62-63	1-Anisyl-1, 3-bis(<i>p</i> -hydroxy- phenyl)propane	<10, 000	(207)

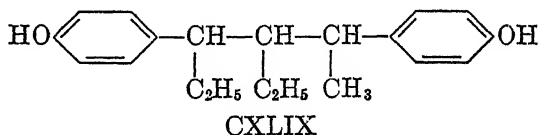
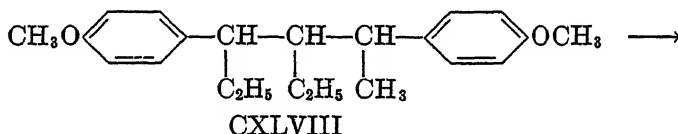
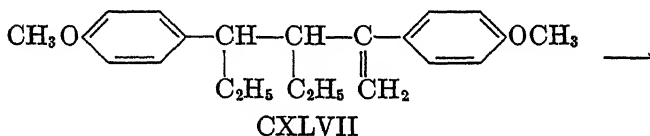
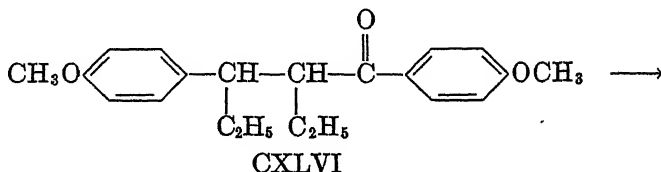
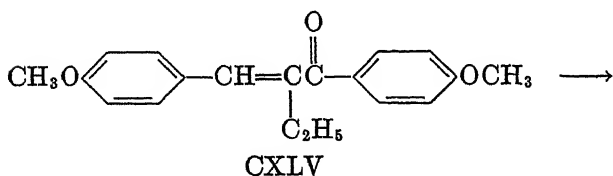
H	CH ₃	H	130	1,3-Bis(<i>p</i> -hydroxyphenyl)-2-methylpropane	<10,000	(207)
H	C ₂ H ₅	H	102	3-(<i>p</i> -Hydroxybenzyl)-4-(<i>p</i> -hydroxyphenyl)butane	<10,000	(207)
H	<i>n</i> -C ₈ H ₁₇	H	118-119	4-(<i>p</i> -Hydroxybenzyl)-5-(<i>p</i> -hydroxyphenyl)pentane	5,000 (70%)	(207)
C ₂ H ₅	H	C ₂ H ₅		3,5-Bis(<i>p</i> -hydroxyphenyl)heptane	1,000 (70%)	(12)
H	C ₂ H ₅	C ₂ H ₅		3-(<i>p</i> -Hydroxyphenyl)-4-(<i>p</i> -hydroxybenzyl)hexane	15 (70%)	(12)
C ₂ H ₅	CH ₃	H	123	3,5-Bis(<i>p</i> -hydroxyphenyl)-4-methylpentane		(25)
C ₂ H ₅	H	C ₂ H ₅		3,5-Bis(<i>p</i> -hydroxyphenyl)heptane	1,000 (70%)	(12)
CH ₃	C ₂ H ₅	CH ₃		2,4-Bis(<i>p</i> -hydroxyphenyl)-3-ethylpentane	10 (70%)	(12)
CH ₃	<i>n</i> -C ₈ H ₁₇	CH ₃		2,4-Bis(<i>p</i> -hydroxyphenyl)-3-propylpentane	8 (70%)	(12)

TABLE 16—Concluded

FORMULA 				NAME	ESTROGENIC ACTIVITY IN RATS SURVIVORSHOUSLY (100 PER CENT RE- SPONSE UNLESS INDICATED OTHERWISE): MINIMUM EFFECTIVE DOSE	REFERENCES
R	R'	R''	MELTING POINT °C.			
CH ₃	C ₂ H ₅	C ₃ H ₇	°C.	2,4-Bis(<i>p</i> -hydroxyphenyl)-3-ethylhexane (mixture of four racemates)	3 (70%)	(12, 206)
			Resin	Racemic mixture "A-1"	10 (70%)	(12, 206)
			75	Racemic mixture "A-2"	5 (70%)	(206)
			144	Racemic mixture "B-1"	85 (70%)	(206)
			162	Racemic mixture "B-2" ("ben-zestrol")	0.8 (70%)	(12, 206)
			Oil	Diacetate	5	(206)
			Oil	Dipropionate	7.5	(206)
			Oil	Dibutyrate		(206)
			Oil	Divalerate		(206)
			Oil	Dilaurate	100	(206)
CH ₃	C ₂ H ₅	C ₃ H ₇	Oil	Dicaprylate	40	(206)
			88	Dipalmitate	200	(206)
CH ₃	<i>n</i> -C ₈ H ₁₇	C ₂ H ₅	118	Dibenzonate	50	(206)
				2,4-Bis(<i>p</i> -hydroxyphenyl)ethyl-heptane	15	(12)
				2,4-Bis(<i>p</i> -hydroxyphenyl)-3-propylhexane	12	(12)
				1,3-Bis(<i>p</i> -hydroxyphenyl)-2-methyl-1-pentene ("dianol")	100 1,000	(23) (25)
			Resin	1,5-Bis(<i>p</i> -hydroxyphenyl)pentane	100,000	(105)

	1, 6-Bis(<i>p</i> -hydroxyphenyl)hexane	Inactive	(6, 25)
	1, 4-Bis(<i>p</i> -hydroxybenzyl)butane	10, 000	(25)
	3, 4-Bis(<i>p</i> -hydroxybenzyl)hexane	Inactive at 50	(6)
	1, 4-Bis(<i>p</i> -hydroxyphenyl)hexane	5, 000	(25)
	1-(<i>m</i> -Hydroxyphenyl)-3-(<i>p</i> -hydroxyphenyl)pentane		(90)
	1-(<i>m</i> -Hydroxyphenyl)-3-(<i>p</i> -hydroxyphenyl)hexane	20, 000 (70%)	(12, 90)
	1-(<i>p</i> -Hydroxyphenyl)-3-(<i>o</i> -hydroxyphenyl)butane		(90)
	1-(<i>p</i> -Hydroxyphenyl)-3-(<i>o</i> -hydroxyphenyl)hexane		(90)
	1-(<i>o</i> -Hydroxyphenyl)-3-(<i>p</i> -hydroxyphenyl)hexane		(90)

none (XXXIII). A series of Grignard reactions effects introduction of the second ethyl group to CXLVI and of the methylene group to CXLVII. Hydrogenation to CXLVIII and demethylation complete the synthesis.

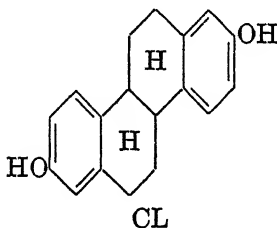


The end product is a resinous mixture of four theoretically possible racemates and is active at the 3-microgram level. From this complex mixture individual racemates have been isolated, though more conveniently by means of a preliminary separation of the two racemic modifications of the ketone (CXLVI). Each racemate was then carried through the remaining reactions, resulting in mixtures of only two racemic mixtures. The relative amounts of the two isomers produced in each case could be varied somewhat depending on the conditions (yet unpublished) of hydrogenation. As shown in table 16, the four racemic mixtures vary considerably in potency. The most active one was called "octofollin" or "benzestrol" and is fully active in doses of 0.8 microgram. Various esters have been prepared which again show prolonged activity but require increased dosage. The series was recently supplemented by position isomers regarding hydroxyl groups; for these substances so far only the bactericidal activity has been reported by Heinemann (90).

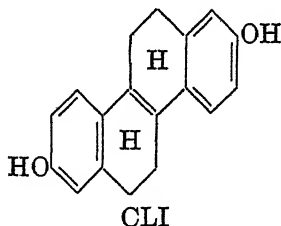
IX. RING-CLOSED ANALOGS

It has been shown how the development of synthetic estrogens began with condensed ring systems, whence it led to the simple hydroxystilbene derivatives. Variation of the stilbestrol structure prompted once more the investigation of condensed ring systems with the stilbestrol skeleton as part of their structure (table 17).

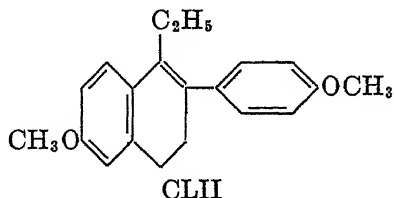
Stilbestrol, written in the *trans*-form, is suggestive of ring-closed analogs of three structural types: chrysene, phenylnaphthalene, and phenylindene. Compounds representing the first two were prepared in the early stage of their work by Dodds, Goldberg, Lawson, and Robinson (59) without, however, encountering highly active estrogens. *trans*-2,8-Dihydroxy-5,6,11,12,13,14-hexahydrochrysene (CL)



is active only in doses of 1 mg. Salzer (175) synthesized the diacetate of 2,8-dihydroxy-5,6,11,12-tetrahydrochrysene (CLI). Introduction of the 11,12



double bond results in a greatly increased activity of 10 micrograms. Dodds *et al.* (59) further obtained 1-ethyl-2-(*p*-anisyl)-3,4-dihydro-6-methoxynaphthalene (CLII), but demethylation resulted in the corresponding 1,2,3,4-tetrahydro



derivative, which is active only at the 10-mg. dose level. The reductive demethylation, possibly by disproportionation, recalls the formation of hexestrol by demethylation of anethole.

TABLE 17
Synthetic estrogens with condensed ring systems

FORMULA	MELTING POINT	NAME	ESTROGENIC ACTIVITY IN RATS (*IN MICE) SUBCUTANEOUSLY (100 PER CENT RESPONSE UNLESS INDICATED OTHERWISE): MINIMUM EFFECTIVE DOSE	REFERENCES
	193 °C.	1-Methyl-2-(p-hydroxyphenyl)-3,4-dihydro-6-naphthol	micrograms 0.3-0.5 0.2	(175) (174)
	256	1-Ethyl-2-(p-hydroxyphenyl)-1,2,3,4-tetrahydro-6-naphthol	10,000	(59)
		3,9-Diacetyl-5,6,11,12-tetrahydrochrysene	100	(175)
	263-264	<i>trans</i> -4,10-Dihydroxy-1,2,7,8,13,14-hexahydrochrysene	1,000	(58, 59)

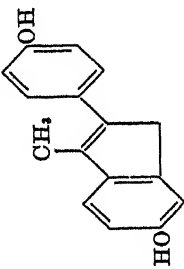
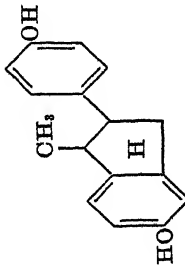
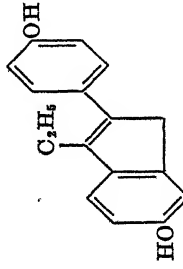
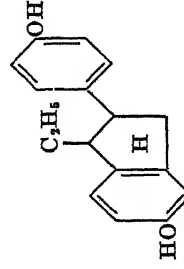
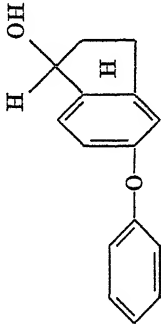
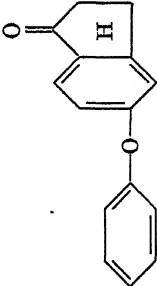
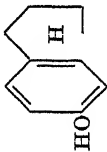
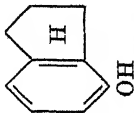
	131	2-(<i>p</i> -Hydroxyphenyl)-3-methyl-6-hydroxy-2,3-indene Diacetate	0.3-0.5 0.2	(175) (175) (174)
	Liquid Liquid	2-(<i>p</i> -Hydroxyphenyl)-3-ethyl-6-hydroxyindane Diacetate	Inactive at 200	(175) (175)
	136 118-120 88-89	2-(<i>p</i> -Hydroxyphenyl)-3-ethyl-6-hydroxy-2,3-indene Diacetate Dipropionate	1.2 (50%)* 0.9 (50%)* 30 (50%)*	(194) (194) (194)
	162-163	2-(<i>p</i> -Hydroxyphenyl)-3-ethyl-6-hydroxyindane	1.8 (50%)*	(194)

TABLE 17—Continued

FORMULA	MELTING POINT	NAME	ESTROGENIC ACTIVITY IN RATS (IN MICE) SUBCUTANEOUSLY (100 PER CENT RESPONSE UNLESS INDICATED OTHERWISE); MINIMUM EFFECTIVE DOSE	REFERENCES
	111 °C.	6-Benzoxo-1-indanol	<i>micrograms</i> Inactive at 99	(137)
	141	6-Benzoxo-1-indanone		(137)
	55	6-Hydroxyindane	Inactive	(64)
	244–246	7-Hydroxyindane	Inactive	(64)

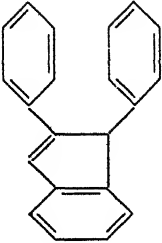
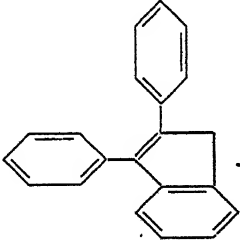
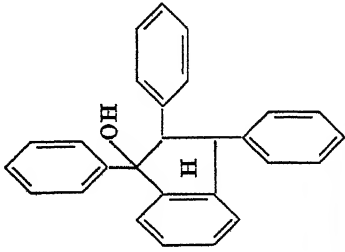
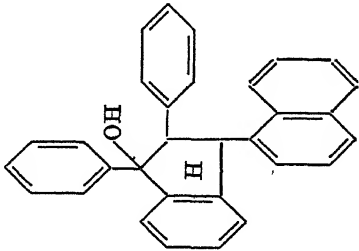
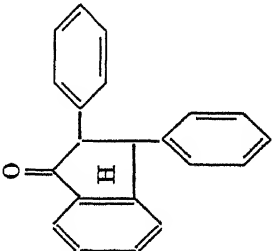
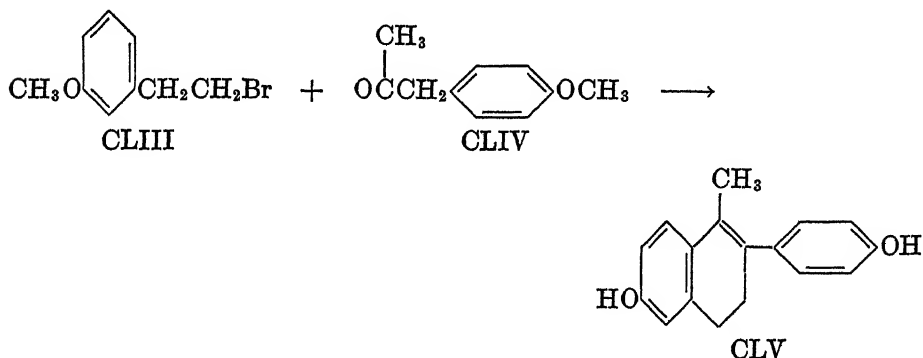
	1,2-Diphenylindene	20 mg. per 20-g. rat	(5)
	2,3-Diphenylindene	20 mg. per 20-g. rat	(5)
	1,2,3-Triphenyl-1-indanol	100,000	(130)

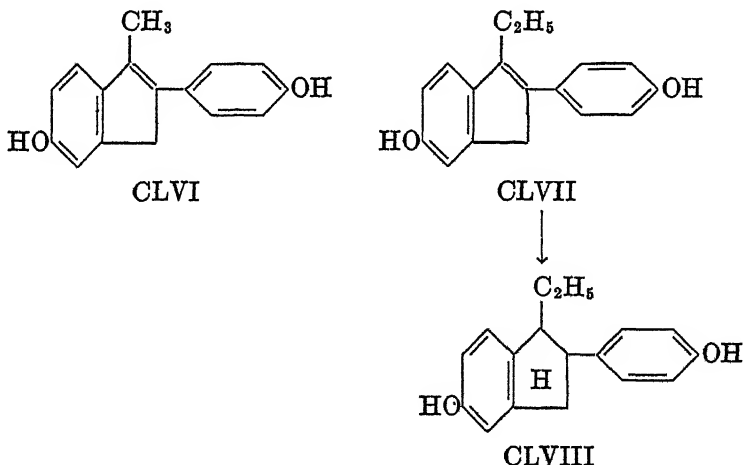
TABLE 17—Concluded

FORMULA	MELTING POINT	NAME	ESTROGENIC ACTIVITY IN RATS (*IN MICE) SURCUTANEOUSLY (100 PER CENT) RESPONSE UNLESS INDICATED OTHERWISE); MINIMUM EFFECTIVE DOSE	REFERENCES
	°C.	1-α-Naphthyl-2,3-diphenyl-1-indanol	micrograms 100,000	(130)
		2,3-Diphenyl-1-indanone	100,000	(130)

Salzer (174, 175) succeeded in preparing the desired phenolic dihydro derivative of the next lower homolog (CLV) in a three-step synthesis from 2-(*m*-methoxyphenyl)ethyl bromide (CLIII) and *p*-anisylacetone (CLIV), followed by ring closure and demethylation; the resulting 1-methyl-2-(*p*-hydroxyphenyl)-



3,4-dihydro-6-hydroxynaphthalene (CLV) was reported active in the order of stilbestrol. In an analogous manner this worker condensed (*p*-anisyl)acetone with *m*-methoxybenzyl chloride followed by ring closure and demethylation, to give 2-(*p*-hydroxyphenyl)-3-methyl-6-hydroxy-2,3-indene (CLVI).

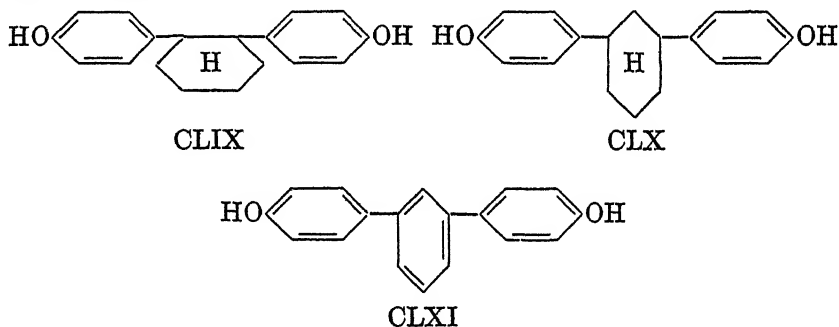


Its diacetate possesses the same activity as the dihydronaphthalene derivative (CLV). Salzer found that the indene-ring closure by means of sulfuric acid could not be effected when the methyl group was replaced by an ethyl group. Solmssen (194) obtained this ethyl homolog (CLVII) by a different method and found its diacetate (C) to be $\frac{1}{12}$ as active as stilbestrol. Incidentally, the 6-position of one of the hydroxyl groups has not yet been strictly proven for either the methyl or the ethyl derivative. Salzer found that catalytic reduction of the indene (CLVI) resulted in a dihydro derivative inactive in doses as high as 200

micrograms. The low activity of this indane, as well as that of the hexahydrochrysene (CL), led Salzer to conclude that disappearance of the "stilbenoid" double bond in cyclic analogs reduces the activity to 1/1000 of that of the unsaturated compound. This conclusion is certainly not justified, because the indane (CLVIII), prepared by hydrogenation of the indene (CLVII), is not less than one-half as active as the latter (194). Plentl and Bogert (150) undertook model experiments for the synthesis by a third method of similar phenylindene and phenylnaphthalene derivatives. So far these authors have described only compounds lacking the phenolic hydroxyl groups in the molecule. For feeble estrogenic activity these are possibly not essential, because Badger *et al.* (5) described some active hydrocarbons, and Monche and Monguio (130) some active indane derivatives with hydroxyl groups in the cyclopentane ring only.

Mentzer and Urbain (123) synthesized compound CLV by a modification of the methods employed by Salzer and by Dodds.

Price and Mueller (154) attempted unsuccessfully to synthesize 1,2-bis(*p*-hydroxyphenyl)cyclohexane (CLIX), which might be considered a ring-closed analog of hexestrol.



The Friedel-Crafts reaction between 1,2-dichlorocyclohexane and anisole proceeded with rearrangement to the 1,3-isomer (CLX), and yielded further 4,4'-dihydroxy-*m*-terphenyl (CLXI); both compounds were found to be inactive. Several cyclohexane derivatives with phenolic rings attached to the same carbon atom are listed in table 15 under cyclic diphenylmethane derivatives.

X. RELATION OF SYNTHETIC ESTROGENS TO CARCINOGENIC SUBSTANCES

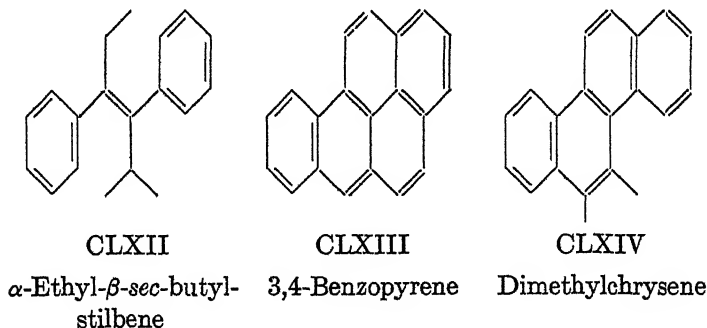
The early work of Cook and Dodds on synthetic estrogens (32, 35) originated from the interest in the problem of a possible correlation between estrogenic and carcinogenic effect. The then somewhat unexpected estrogenic activity of 5,6-cyclopenteno-1,2-dibenzanthracene and 1,2-benzopyrene appeared at first to be significant. Later it was seen that the low order of potency of these compounds is shared by many other non-carcinogenic substances. Subsequent years brought the further development of synthetic carcinogenic hydrocarbons and of phenolic estrogens as two independent lines of research which, to this date, have added little to the understanding of the influence of female sex hor-

mones on the transformation of normal into cancerous tissue. Nevertheless, synthetic as well as natural estrogens are rapidly gaining in importance for the treatment of certain malignant diseases. The discussion of the rationale of this therapy is beyond the scope of this review. The earlier work has been treated by Fieser (77) and the more recent developments by Dodds (53) and by Haddow *et al.* (88).

TABLE 18
Carcinogenic effectiveness of estrogens (on injection, in mice)

ESTROGENS	(a) ESTROGENIC DOSE (50 PER CENT RESPONSE) IN MICROGRAMS	(b) CARCINOGENIC DOSE		(c) RATIO (b):(a)
		Milligrams	Response	
Estrone.....	1.0	32.8	100%	32.8
Estradiol.....	0.3	25.0	50%	83.3
Estradiol benzoate....	0.6	0.6	100%	1.0
Stilbestrol.	0.3	18.2	100%	60.8
Stilbestrol monomethyl ether..	2.0	9.5	50%	4.8
Stilbestrol dimethyl ether..	25.0	116.5	50%	4.6

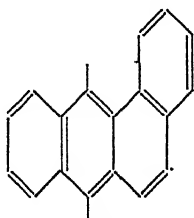
Dodds, Lawson, and Williams (65) selected α -ethyl- β -*sec*-butylstilbene (CLXII) as a stilbene derivative closely resembling 3,4-benzopyrene (CLXIII) and dimethylchrysene (CLXIV).



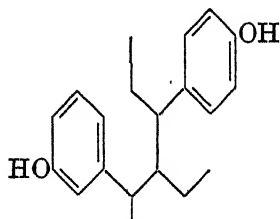
When painted on mice, this stilbene derivative proved to be carcinogenic, while no positive results were obtained with diphenylhexane, diphenylhexadiene, diethylstilbene, stilbestrol, hexestrol, and 4,4'-dihydroxystilbene.

Employing another technique, Geschickter and Byrnes (85) produced mammary cancer in mice with stilbestrol and some derivatives. Their data, reproduced in table 18, are of particular interest because they include parallel experiments with some natural estrogens. Similar results had been obtained earlier by Shimkin and Grady (189), and it appears that there may be a quantitative, but no qualitative, difference between synthetic and natural estrogens in their relation to the production of cancer. Martin (118) called attention to the similarity of 9,10-dimethyl-1,2-dibenzanthracene (CLXV), one of the most

active carcinogenic hydrocarbons known, and benztrol (CXLIX) when the latter is written as shown in CLXVI.



CLXV

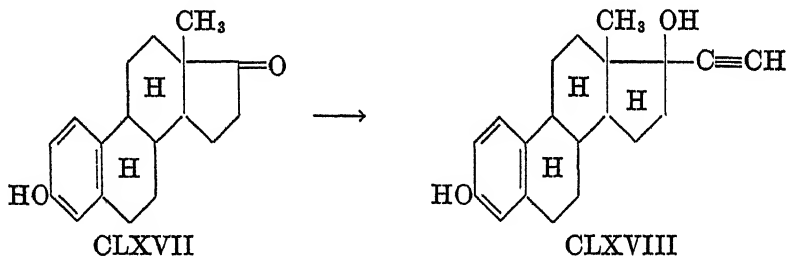


CLXVI

However, the postulated carcinogenic effectiveness of benztrol has not been corroborated by experimental evidence (206).

XI. METABOLIC CONVERSIONS AND EFFICACY BY VARIOUS ROUTES

Whereas the subcutaneous activity provides the most convenient yardstick for comparing various estrogens, the comparative efficacy by different routes is of physiological interest. Moreover, the high oral activity of synthetics is important from a practical point of view. For the benefit of the subsequent discussion it is proposed to include ethinylestradiol (CLXVIII) among the synthetic estrogens.



CLXVII

CLXVIII

Inhoffen *et al.* (94) prepared this compound by the action of potassium acetylide on estrone (CLXVII), and reported its especially high oral activity. Today it is recognized as probably the most active estrogen known. As a synthetic conversion product of a natural estrogen it represents an intermediate type, but for reasons apparent in the course of the following discussion it is more appropriate to classify it with the synthetic estrogens.

The oral efficacy of synthetics is relatively and absolutely superior to that of the natural hormones. Zondek and Sulman (234) advanced the theory that this is due to differences in the resistance of the products to metabolic degradation. After rats had been injected with estrogenically equivalent amounts of estrogens, stilbestrol was recovered in 25 per cent from the body and in equal amounts from the excreta; the esters of natural estrogens behaved similarly, while only 1 to 2 per cent of the administered estrone was found in the body and none in the excreta. Silberstein and coworkers (191) had previously shown *in vitro* that estrone is inactivated by liver tissue as well as by blood of dogs. Consequently it was generally accepted that the process of estrone inactivation took place in the liver, possibly involving an enzyme, and that the stability of stil-

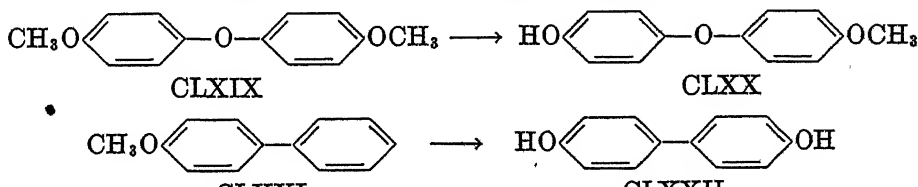
bestrol within the organism was due to the inability of the liver to inactivate the synthetic compound. Later this interpretation had to be modified when more experimental evidence became available. Dingemanse and Tyslowitz (48) found an appreciably higher urinary excretion of estrone than reported previously. Zondek *et al.* (235) investigated the *in vitro* inactivation of stilbestrol and reported that rat liver pulp inactivates it also, though less readily than the natural estrogen. According to these workers the susceptibility to inactivation is the same if the two types of estrogens are compared on the basis of their estrogenic activity. Chamelin and Funk (233), applying this finding, reported that the mortality of rats due to excessive doses of stilbestrol could be significantly lowered by the concurrent administration of liver extract.

The hypothesis of enzymatic inactivation was elaborated by Westerfeld (227), reasoning that estrogens, like other phenols, might be metabolized by oxidation to *o*- or *p*-dihydrophenols and subsequently to the corresponding quinones. The enzyme tyrosinase effects such oxidations and, applying it to estrogens, Westerfeld showed that estrone, estradiol, and stilbestrol are inactivated *in vitro*, the latter in amounts exceeding 75 per cent. This author recognized that these results are inconclusive without the isolation of the postulated inactivation products and, in fact, no such products have been encountered among the excretion products isolated by others (119, 205). Moreover, tyrosinase has not been encountered in mammalian tissue and thus the above experiments do not elucidate the mechanism of inactivation *in vivo*.

The fate of estrogens after parenteral administration has been the subject of further investigations. Stroud (203) made a quantitative study of the recoveries of various estrogens from the urine of rabbits. For the first time the excretion products of synthetics were isolated in crystalline form and in amounts corresponding to the total estrogenic activity of the urine. One half of the total recovered stilbestrol was present as conjugate, the other half free.

Mazur and Shorr (119) identified the conjugate as the glucuronide, with stilbestrol combined in glucosidic linkage. Up to 30 per cent of the administered amount was recovered as crystalline conjugate, and the authors concluded that "its magnitude indicates the significance of this conjugating mechanism for the intermediary metabolism of stilbestrol."

Stroud (205) contributed experimental evidence for the dealkylation of estrogenic phenol ethers in the organism of female rabbits. Thus 4,4'-dimethoxydiphenyl ether (CLXIX) was physiologically converted into the monohydroxy-monomethoxy derivative (CLXX) and 4-methoxydiphenyl (CLXXI) into 4-hydroxy- and 4,4'-dihydroxy-diphenyls (CLXXII). Only the last compound is estrogenically active. This was the first observed instance of the biological production of an active substance from inactive material. The same worker



(202) extended the investigation of excretion products to other active and inactive phenols previously assayed by Dodds *et al.* (59, 64).

In all cases (shown in table 19) phenolic products were formed, resulting in activation rather than inactivation of the parent compounds. However, only the stilbenes, active on their own account, gave diphenols of significant activity. Stroud noted that the yield of recovered phenols seemed to be inversely proportional to the estrogenic activity of the parent compound and of the phenol produced. The metabolic conversion product of diphenylhexadiene (CLXXIX) was obtained in minute amounts only, but its identity with dienestrol (XXI) has been made very probable.

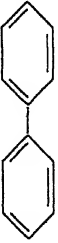


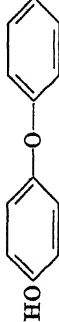






This evidence for physiological transformation into *p*-hydroxy derivatives may explain the lower activity of the ortho- and meta-substituted estrogens, as shown in table 9. Compounds with one hydroxyl group in the para-position, or even without any, may be biologically activated by conversion into *p,p'*-substituted substances. On the other hand, in compounds with hydroxyl groups in the ortho- or meta-position no additional phenolic groups in the para-position need to be introduced for conjugation and elimination from the organism.

The problem of the true or precursor nature of various compounds has been the subject of an interesting treatise by Emmens (75), who derived the classification into two groups of "true" estrogens and of "pro-estrogens," based on the striking difference in the subcutaneous and intravaginal potency as shown by Freud (84). Emmens reasoned that in the case of intravaginal application only the "true" estrogen would act directly on the vagina and cause cornification of the mucosa, while "pro-estrogens" would be absorbed from the vagina into the circulation as from injection elsewhere, converted into an active metabolite, and returned to the vagina in no greater dose or concentration than available after subcutaneous injection.

Previously, Robson and Adler (163) and also Morell and Hart (201) had shown that estrogens may act locally on the vaginal mucosa without appreciable absorption into the general circulation. The former authors constructed in mice a separate vaginal pocket from the lower vagina; the administration of 0.2 microgram of stilbestrol into one part of the vagina produced there complete cornification while leaving the other, separated part unaffected. Similar results were obtained with natural estrogens and their esters.

Emmens then determined the ratio (S/L) of the systemic to the local dose for a large number of synthetic and natural estrogens, and this led to the classification into two clearly differentiated groups: "true estrogens" with S/L in no case less than 50 and "pro-estrogens" with S/L in no case more than 2. Among the compounds listed as "true estrogens" are estrone, estradiol, estriol, ethinyl-estradiol, stilbestrol, ψ -stilbestrol, hexestrol, isohexestrol, dienestrol, and esters of natural and of synthetic compounds. The group of "pro-estrogens" includes only synthetic compounds of low activity like 4-hydroxydiphenyl (CLXXIV), 4,4'-dihydroxydiphenyl (IX), 4-hydroxydiphenyl ether (CLXXVI), stilbene (X), 4-hydroxystilbene, 4,4'-dihydroxystilbene (XI), 4-hydroxy- α,β -diethylstilbene (CVII), dihydroxyhexahydrochrysene (CL), and 9,10-di-*n*-propyl-9,10-

TABLE 19

PARENT COMPOUND	PHENOLIC METABOLIC PRODUCT	TOTAL RECOVERY† per cent
 CLXXIII (inactive)*	 CLXXIV (inactive)	25.4
 CLXXV (inactive)	 CLXXVI (100 mg.)	22.8
 CLXXVII (inactive)	 CLXXVIII (inactive)	14.7
 X (25 mg.)	 XI (5 mg.)	2.9
 CLXXIX (10 mg.)	 XXI (0.4 microgram)	

* "Inactive" infers inactivity in rats at 100-mg. dose level.

† Total recovery includes "free" and "combined" phenols.

dihydroxy-1,2,5,6-dibenzanthracene (III). Emmens classified triphenyl-chloroethylene (CXXVIII) among the true estrogens, but Robson *et al.* (189) reported that the closely related α,α -bis(*p*-ethoxyphenyl)- β -bromo- β -phenylethylene (CXXIX) is a pro-estrogen by Emmens definition, and the same result was obtained by Thompson and Werner (210c) for β -tri(*p*-anisyl)- β -chloroethylene.

Emmens remarked that there is no rigid proof regarding the soundness of his theory, but he cited the sharp differentiation of S/L values close to unity and those above 50 as an argument in favor of it. On the other hand, the S/L values within the group of "true" estrogens vary from 50 to 3000, raising some doubt as to the weight of Emmens' argument. Obviously S/L will be greatly affected by relatively minor changes of either systemic or local efficacy, both depending on factors distinct from estrogenic activity, like the rate of absorption or the stability under local conditions. For example, Muhlbock (242) demonstrated that the increased action on intravaginal application was further increased by administration in 50 per cent aqueous glycerine.

As mentioned before, the group of "true" estrogens includes the various esters of natural as well as of synthetic compounds. Emmens remarked that the S/L ratio is raised for the esters owing to an increase of the subcutaneous dose, while the intravaginal dose, on a molar basis, is little different from that of the parent compounds. This is explained by the assumption of a high degree of efficiency of the vaginal mucosa for ester hydrolysis. Emmens postulated that "physiological doses of substances which are active locally are fixed or utilized in the tissues, and do not escape in appreciable amounts into the circulation, whereas pro-estrogens are not immediately utilized or fixed, but pass into the circulation and undergo metabolic changes to estrogens." The same author (74) later confirmed experimentally that "true estrogens" administered into one part of the separated vagina, even in amounts exceeding the locally effective dose, do not stimulate the second part, while "pro-estrogens" stimulated both parts when introduced into one of them. These findings add weight to the arguments in favor of the classification into "true" and precursor estrogens, and it is significant that on purely biological grounds, the highly active synthetic estrogens are classified together with the natural hormones.

Segaloff (217) undertook further investigations of the mechanism of estrogen inactivation in the living organism. In rats various estrogens were injected into the spleen transplanted to a subcutaneous position without portal drainage to the liver, and into the spleen *in situ*. The ratio of the minimal effective dose for *in situ* injection and the dose for injection into the transplanted spleen is interpreted as a measure for the degree of inactivation taking place in the liver. For stilbestrol and hexestrol the ratio is about 20, which indicates that these compounds are inactivated, but to a lesser extent than estrone and estradiol, as shown previously by the same author. Esterification protects stilbestrol to a certain extent (ratio 3.5), but complete protection (ratio 1) is only achieved by alkylation. Benzestrol (CXLIX) is subcutaneously active at a higher threshold than stilbestrol, but it is inactivated to a lesser degree (ratio 10). That the mechanism of

TABLE 20

Comparative oral activities

(a) Minimum effective dose in micrograms (100 per cent response unless indicated otherwise); (b) oral dose as multiple of subcutaneous dose

STILBESTROL		HEXESTROL		DIENESTROL		ESTRONE		ESTRADIOL		ETHINYLESTRADIOL		REFERENCE
(a)	(b)	(a)	(b)	(a)	(b)	(a)	(b)	(a)	(b)	(a)	(b)	
Rats												
3	(50%)	7.5		3	7.5							(91)
5				3								(59)
5		4.5				70	20					(221)
2.75	(50%)	1.6				225 (50%)	75	175 (50%)	97			(197)
5		4										(156)
1.35		10				4	0.8	4	16			(104)
0.3		3-6										(183)
Mice												
0.35	(50%)	5				9 (50%)	105	7.5 (50%)	500			(197)
0.38	(50%)	4.75										(127)
0.4	(50%)	4		0.1 (50%)	1	1 (50%)	10	1.1 (50%)	45	1.2 (50%)	60	(73, 166)
0.5	(50%)	5				25	35					(72, 104)

benzestrol inactivation may be different is indicated by the practically identical ratio for its diacetate. Ethinylestradiol (CLXVIII) has the high ratio 42, while the subcutaneous threshold dose of 0.18 microgram (for 50 per cent response) is low compared with 0.33 for stilbestrol or hexestrol. Segaloff concluded that the high oral activity of ethinylestradiol is due not to its greater resistance to hepatic inactivation but indeed to its greater potency. The triphenylethylene derivatives and the substituted dibenzanthracene (III) believed to be "pro-estrogens" show an entirely different ratio. Their activity is enhanced by passage through the liver, where chemical changes take place resulting in the formation of more active compounds.

This last study demonstrates that high oral efficacy may be the composite result of various factors, among them the susceptibility to hepatic degradation and the structural conditions favorable for the biological conversion into more active compounds. Probably most important are specific structural requirements like the phenolic hydroxyls and the six-carbon chain of stilbestrol and its analogs, or the ethinyl group in ethinylestradiol.

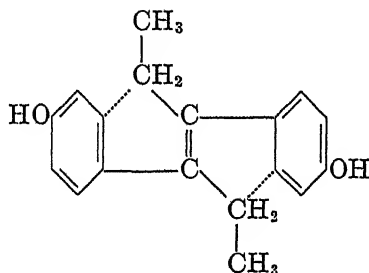
In order to give a rough estimate of comparative oral activities, though by no means a complete evaluation, those of some of the more prominent compounds have been summarized in table 20. Considerable disagreement between the figures from various origins and a marked species difference will be noted. The duration of estrogenic effect after subcutaneous administration has been discussed earlier; the situation is quite different regarding oral administration. Kreitmair and Sieckmann (104) and Emmens (72) agreed that prolongation of effect due to esterification of stilbestrol and hexestrol does not occur on oral application. Both, parent substances and their esters, give only a short lasting effect and very large amounts of certain esters, especially the dipropionate, are required to achieve moderate prolongation. Undoubtedly, the main factor involved in the prolonged effect of these compounds is the delayed resorption from the site of injection, while in the case of oral administration hydrolysis might occur in the gastric tract or the larger amount of administered estrogens may be excreted without resorption taking place. The situation is different with estrogens of the triphenylethylene series. Robson and Schönberg (166, 167) and Davies *et al.* (39) showed that the prolonged effect of these compounds and the regulation of duration by proper dosage is obtained also on oral administration.

The investigations discussed above do not yet give a complete picture of the mechanism of estrus production and biological inactivation, apparently both being related to each other. Nevertheless, the study of synthetics and their difference in behavior from the natural estrogens has made a start towards the understanding of the chemical reactions taking place in the course of hormonal action.

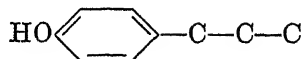
XII. APPENDIX

There have been several attempts to generalize the requirements of chemical structure for a high degree of estrogenic potency.

Campbell (19) called attention to the "possibility of ring-closure of certain synthetics to give compounds containing cyclopentane rings with incidental methyl groups," as shown in formula CLXXX.



CLXXX



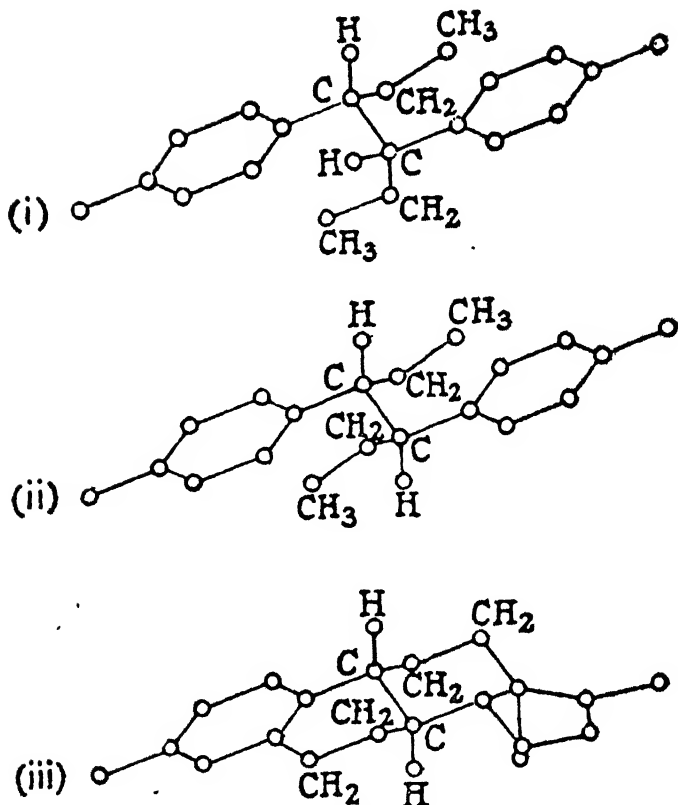
CLXXXI

Linnell and Sharma (114) postulated that the skeleton shown in CLXXXI is essential for estrogenic potency of the highest order. These generalizations in no way explain the lesser activity of isomers identical except for the spatial arrangement. Dodds *et al.* (56) underlined the importance of this spatial arrangement and added that "the disposition of the hydroxyl groups in a molecule of suitable shape may be of greater importance than a close relation to the formula of estradiol as written in one plane." There are too many deviations from the planar structural resemblance to estradiol for this to be convincing. On the other hand, this proved a useful working hypothesis, stimulating much research, though at times it has been abandoned in favor of more empirical approaches. While the structural resemblance between natural and synthetic compounds must not be taken literally, there is also good evidence that the various types of structure, divergent as they may seem, have a great deal in common and that the hormonal activity of the highly potent synthetics is by no means accidental.

Giacomello and Bianchi (86) measured the size of the molecular skeleton of stilbestrol and estrone and found complete agreement, both molecules being 8.55 Å. long and 3.88 Å. wide. No comparative measurements of the molecular size of the less active isomer have as yet been reported.

Carlisle and Crowfoot (27) found a certain similarity between the crystallography of estrone and that of hexestrol (*meso*-form) and of isohexestrol (*dl*-form of 4,4'-dihydroxy- α,β -diethyldibenzyl). Quoting these authors:

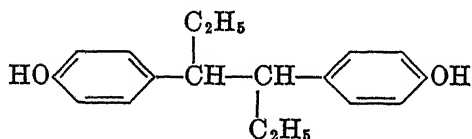
"These similarities probably express little more than the fact that the configurations of the molecules of both diethyldibenzyl series are of an extended form, perhaps that illustrated in the figure [CLXXXII] or of the variety found in dibenzyl itself. This being so, it is an interesting fact that the stereochemical arrangement of the atoms in the *meso*-series" of the diethyl derivative above, "which is biologically the most active series, is very closely related to the stereochemical form deduced for the natural sex hormones. The fact that the *meso*-compounds have a centre of symmetry in the crystals proves that the disposition of the atoms about the central carbon-carbon bond is of the *trans*-type considered characteristic of the junction between rings B and C of the sterol sex-hormone series."



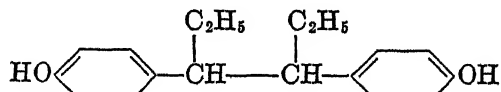
CLXXXII

Diagram (from Carlisle and Crowfoot (27)) to illustrate the relation of the stereochemical forms of the isomers of 4,4'-dihydroxy- α,β -diethyldibenzyl to that probably present in estrone. Possible atomic arrangement in (i) *d*- or *l*-4,4'-dihydroxy- α,β -diethyldibenzyl; (ii) *meso*-4,4'-dihydroxy- α,β -diethyldibenzyl; (iii) estrone.

This interpretation disposes of an apparent discrepancy between the correlation of activity and configuration in the hexestrol and in the stilbestrol series. The planar projection formulas of hexestrol and isohexestrol might seem to indicate that the racemic form (CLXXXIII) rather than the *meso*-form (CLXXXIV) would correspond to the *trans*-configurational formula (XCIV) of stilbestrol.



CLXXXIII



According to Carlisle and Crowfoot both hexestrol and isohexestrol have spatial formulas closely resembling those of estrones, the only difference being the spatial arrangement of one hydrogen atom and one ethyl group, respectively. The hundredfold difference in estrogenic activity demonstrates the importance of this group in its spatial relation to the rest of the molecule.

Regarding the relation between chemical structure and estrogenic potency, some general conclusions may be drawn, though it is recognized that their value is limited in view of the danger of attaching undue weight to the relative potency of various compounds.

The most striking fact is the almost rigid specificity of estrogenic potency regarding the presence and position of the two hydroxyl groups, combined with a considerable lack of specificity regarding the central structure linking the aromatic rings. By analogy with the natural estrogens it might be expected that one of the two hydroxyl groups need not be phenolic, but on the basis of limited evidence available to date, it appears that aromatic hydrogenation with the effect of changing one phenolic group into an alcoholic one causes inactivation. In the stilbene series, additional substituents in the aromatic rings or a shift of the hydroxyl groups from the para-position cause a sharp drop in activity; the hexestrol series appears somewhat less sensitive to variation. The central structure need not be aliphatic, though it is preferably so for highest potency. The peak of activity is usually found for derivatives with a six-carbon-atom chain or, stated more generally, for structures comprising a total of five to seven carbon atoms, or a chain or ring containing two to three carbon atoms, substituted by aliphatic groups containing two to five carbon atoms. The degree of saturation of the central structure is of less importance than the spatial arrangement; as stilbestrol and the two most active synthetics related to it contain one, two, or no aliphatic double bonds. It is difficult to attempt the interpretation of the relative importance of the phenolic hydroxyl groups and the two ethyl groups in stilbestrol and hexestrol, and the combined effect appears to be an important factor. The aliphatic substituents are definitely less specific than the hydroxyl groups. On the other hand, proper substitution of the central structure may be more specific in terms of biological conversion; while it is feasible that a weakly potent compound like α,β -diethylstilbene is hydroxylated in the organism to give highly active metabolic products, there is no evidence for the assumption that the equally feeble 4,4'-dihydroxystilbene (XI) may be rendered more active by biological substitution with the appropriate alkyl groups.

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Supplement¹ (added January 2, 1946)

III. HEXESTROL

A. DISCOVERY

As discussed earlier, the reductive demethylation of anethole (XIII) by means of alcoholic potassium hydroxide led to the discovery of hexestrol. Another example of the reducing properties of alcoholic potassium hydroxide has been reported by Rubin (255).

B. SYNTHESSES (METHODS OTHER THAN HYDROGENATION)

Kharasch, McBay, and Urry (247) devised an ingenious new synthesis of hexestrol dimethyl ether (XXVII). These workers found that diacetyl peroxide may be decomposed to produce free methyl radicals which remove hydrogen atoms from the solvent, resulting in the production of methane and leaving more complicated free radicals, with the tendency for dimerization. When *p*-methoxy-*n*-propylbenzene is used as the solvent, approximately equal amounts of the dimethyl ethers of hexestrol and isohexestrol are formed. On the basis of recovered starting material, the yield was 16 per cent for hexestrol dimethyl ether; in addition, isohexestrol dimethyl ether was obtained in equal yield. By means of the conversion methods discussed earlier, the overall yield of hexestrol dimethyl ether may be substantially increased.

C. OPTICAL ISOMERISM

Peak and Short (239) obtained a patent for the isomerization of the dimethyl ether of isohexestrol into that of hexestrol by heating to 300–350°C. in the presence of either hydrogen sulfide or palladium on carbon.

IV. STILBESTROL

A. SYNTHESSES

4. Syntheses involving retro-pinacolin rearrangements

Adler and Lundin (237) reinvestigated the chemistry of 3,4-bis(*p*-hydroxyphenyl)-3,4-hexanediol (LXIII) and succeeded in separating into two components the pinacol (m.p. 204–206°C.) obtained by Dodds *et al.* (58, 59, 60) from *p*-hydroxypropiophenone. The one melting at 217–219°C. was called "α-pinacol," and the other, melting at 212–214°C., "β-pinacol." Earlier, Hobday and Short (91) had described a compound called "iso-pinacol," m.p. 94–95°C., which they believed to be the optical isomer of Dodds's pinacol because both gave dienestrol (XXI) on dehydration with acetyl chloride. According to Adler and Lundin the α- and β-forms actually represent the optical isomers with structure LXIII, while "iso-pinacol" is an isomer of yet undetermined structure. Their indirect evidence is based on the analogous behavior of the α- and β-forms

¹ The headings and subheadings of the supplement correspond to those used in the main part of the paper.

in their transformation into the pinacolin (LXV) and into dienestrol (XXI). " α -Pinacolin" is active in 100 micrograms and by analogy with the potencies of hexestrol and isohexestrol, it is assumed to represent the *meso*-form, while the " β -pinacolin" (inactive in 1000 micrograms) would represent the racemic form.

C. ANALYTICAL METHODS FOR STILBESTROL DETERMINATION

A new colorimetric method suitable for the quantitative determination of 0.5–2.0 mg. of stilbestrol, hexestrol, and dienestrol has been described by Malpress (249). The procedure makes use of the yellow color developed after nitration in acetic acid solution, followed by neutralization. The isolation of a crystalline dinitro derivative of hexestrol suggests the same nitration mechanism for stilbestrol and dienestrol.

D. HYDROGENATION OF STILBESTROL

2. Hydrogenation of aromatic rings

Hoehn and Ungnade (242) described some derivatives of the hydrogenation products obtained earlier (92) from stilbestrol with Raney nickel under 4000–5000 lb. pressure. Ungnade and Ludutsky (258) extended this work and succeeded in isolating the two complete series with three perhydro-diols, each of the general formula CIII, and the two perhydro-diketones of the general formula CV. Experimental evidence has been presented for the assignment of the *cis-trans* configuration of the hydroaromatic substituents in the *meso* and in the racemic series. The optical configurations of the two series were derived from their relationship to hexestrol (*meso*-form) and isohexestrol (racemic), respectively (table 21).

In an earlier series of Swiss patents (256, 257, 258, 259, 260, 261), perhydro-diols have been described with characteristics substantially the same as listed in table 21.

While isohexestrol may be considered the normal hydrogenation product of stilbestrol, several instances of hexestrol as the hydrogenation product have been cited earlier; to these may be added the Raney nickel hydrogenation of stilbestrol in the presence of its sodium salt (268).

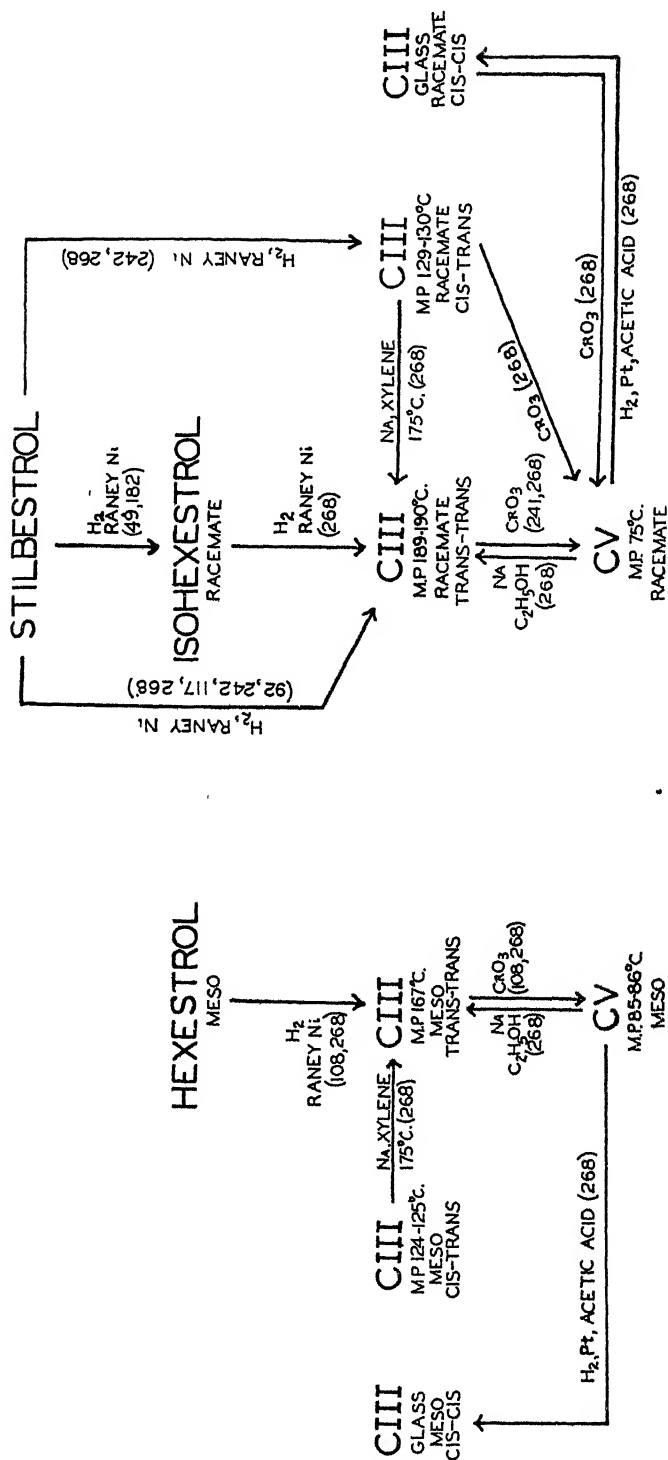
V. VARIATION OF FUNDAMENTAL STRUCTURE

A. VARIATION OF RING SUBSTITUENTS

1. Acylation and alkylation of phenolic hydroxyl groups

Miescher and Meystre (250) found that stilbestrol monosulfate (or phosphate) is advantageously prepared from the monobenzoate, which is transformed into the monobenzoate monosulfate. On heating with sodium carbonate only the organic ester group is hydrolyzed. Reid (252) obtained a patent for the preparation of stilbestrol monoalkyl ethers; the duration of estrus as a function of the dosage is claimed to be a characteristic property of the mono ethers. A similar patent was granted to Schmelkes (262) for hexestrol monomethyl ether.

TABLE 21
Perhydrogenation products¹



¹ The symbols CIII and CV in this table refer to the general structure only.

2. Position isomers and homologs with additional ring substituents

Another effort has been made to obtain, by suitable modification of the stilbestrol molecule, compounds with hormonal activity other than estrogenic. Ross (253) prepared non-phenolic analogs of stilbestrol, hexestrol, and diphenylethane with acetyl groups in place of the para-hydroxyl groups of the estrogens. No information has yet been given regarding the desired luteoid activity of these compounds. Walker (269) undertook earlier to prepare ω -hydroxy methyl ketones having the same side chain as the corticosteroids. Ross (254) made a similar, though unsuccessful, attempt. However, this worker succeeded in synthesizing di(ω -acetoxyacetylphenyl) ether, bis(ω -acetoxyacetyl)diphenyl, and 2,7-bis(ω -acetoxyacetyl)fluorene; all of these showed no marked effect on prolonging the life of adrenalectomized rats.

B. VARIATION OF ALIPHATIC CHAIN

3. Location and number of aliphatic double bonds

Patents for a novel synthesis of dienestrol (XXI) from stilbestrol diacetate (LII) have been granted to Turnbull (266, 267). Bromination of LII in carbon disulfide results in 4,4'-diacetoxy- α,β -dibromostilbene. Subsequent treatment with potassium iodide in alcohol gives a mixture of stilbestrol diacetate (LII) and a product which gives dienestrol on hydrolysis. Dienestrol diacetate (CXVIII) is obtained from the dibromide on refluxing with pyridine. Bromination of hexestrol dimethyl ether (XL) gave 3,4-bis(*p*-anisyl)-3,4-dibromohexane; debromination of the latter was always accompanied by reduction to the starting material (XL).

VI. TRIPHENYLETHYLENE DERIVATIVES

Schönberg and Tadros (263) obtained a patent for the preparation of di(*p*-ethoxyphenyl)benzylcarbinol, presumably as an intermediate in the synthesis of α,α -bis(*p*-ethoxyphenyl)- β -phenyl- β -bromoethylene ("D.B.E.") (CXXIX).

Basford (238) described mixed ethyl methyl ethers related to CXXIX and CXXX.

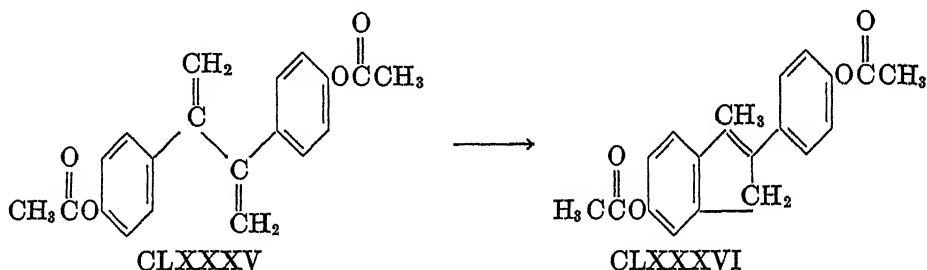
VIII. DIPHENYLPROPANE DERIVATIVES

Stuart *et al.* (265) reported additional dialkyl substitution products of the aliphatic chain in 1,3-bis(*p*-hydroxyphenyl)propane. In the series of 1,3-dialkylpropane derivatives the highest activity of 200 micrograms lies with the methyl propyl compound. Higher activities were found in the 1,2-dialkyl series, with the optimum of 30 micrograms for one of the two racemic 1-ethyl-2-*n*-propyl derivatives.

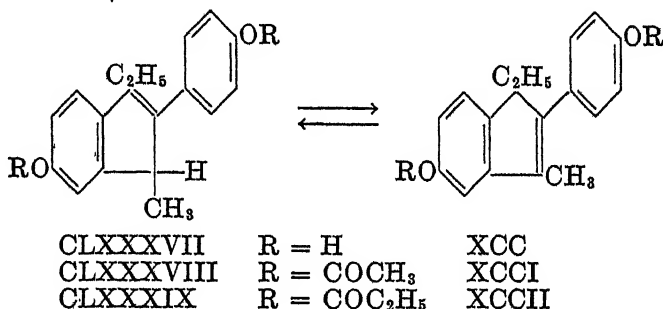
IX. RING-CLOSED ANALOGS

Adler and Hagglund (236) investigated the cyclization of dienestrol, isodienestrol, and its lower homolog, 2,3-bis(*p*-hydroxyphenyl)-1,3-butadiene, as

well as of their diacetates. Cyclization with boron trifluoride did not lead to the tetracyclic succindane derivatives expected by these workers, but to 2-phenylindenes. The structure of the cyclization products appears to be firmly established, because in the case of CLXXXV the reaction product (m.p. 133–134°C.) is 2-(*p*-acetoxyphenyl)-3-methyl-6-acetoxy-2,3-indene (CLXXXVI), i.e., the diacetate of CLVI synthesized by Salzer (174, 175) by another route.

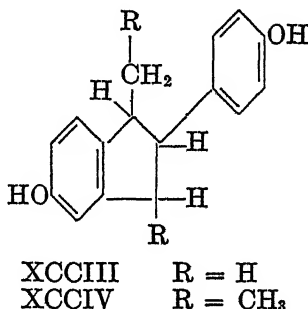


Adler and Hagglund found CLXXXVI inactive in doses of 100 micrograms (in rats, 50 per cent response), while Salzer reported an activity of 0.2–0.3 microgram. This discrepancy is no reason to doubt the identity of the two reaction products, because the inactivity of CLXXXVI at the 60-microgram level (in rats, 50 per cent response) has been confirmed by this reviewer (264) with a preparation synthesized by another method (194). Moreover, the ultraviolet-absorption spectrum of CLXXXVI, as reported by the Swedish workers, conforms closely to the spectrum (194) of the next higher homolog (CLVII). The cyclization of dienestrol (XXI) and of its stereoisomer isodienestrol led to the same reaction product (CLXXXVII), 1-methyl-2-(*p*-hydroxyphenyl)-3-ethyl-6-hydroxy-2,3-indene, m.p. 175°C.



Similar ring closures were carried out with the diacetate (CXVIII) and the dipropionate of dienestrol, resulting in the diacetoxyindene (CLXXXVIII) and the dipropoxyindene (CLXXXIX). Adler and Hagglund observed that in the presence of pyridine or other bases these 2,3-indenes are converted into isomeric esters, most likely having the structure of the 1,2-dienes (XCI and XCCII). This conversion is reversible. The phenolic 2,3-indene (CLXXXVII) could not be converted under the same conditions, but hydrolysis of the esters XCCI and XCCII gave the corresponding phenolic 1,2-indene

(XCC), m.p. 128–129°C. Whether the 2,3-indene structure has been assigned correctly to the higher melting isomer or should be assigned to the lower melting form can not yet be decided definitely; both compounds have practically identical absorption spectra. The interrelationship of the two isomers is undoubtedly correct, because on hydrogenation both absorb one mole of hydrogen and give the same indane derivative (XCCIV).



Similarly, the indene CLXXXVI was hydrogenated to the indane XCCIII. These indanes are stable, while the indenenes are quite generally stable only in the form of their esters (174, 175, 194, 236). Both indanes (XCCIII and XCCIV) are inactive in doses smaller than 100 micrograms, while both 1-methyl-2-(*p*-hydroxyphenyl)-3-ethyl-6-hydroxyindenenes (CLXXXVII and XCC) show the remarkably high potency of 1 microgram (in rats, 100 per cent response). The diacetate CLXXXVIII has the same activity, while the diacetate XCCI and the dipropionate XCCII are slightly less active.

Finally, the fact that Adler and Hagglund, starting from the symmetrical diene LXXXV, obtained the same indene derivative (CLXXXVI) as Salzer (174, 175) and Solmssen (194, 264) by two different routes, provides the structure proof regarding the 6-position of one of the hydroxyl groups in CLVI, CLVII, and CLVIII.

XI. METABOLIC CONVERSION AND EFFICACY BY VARIOUS ROUTES

Lipschutz and Quintana (248, 251) studied the inactivation of estrogens in the form of intrasplenic or intrahepatic pellets. These workers confirmed the observation that inactivation of synthetics takes place but to a lesser degree than with natural estrogens. Engel and Rosenberg (240) also corroborated the enzymatic inactivation of stilbestrol *in vitro*. While Zondek *et al.* (235) had found stilbestrol more resistant against the inactivating liver enzyme than estrone, these workers observed aqueous acid liver extracts to inactivate both types of estrogens equally well *in vitro*. Alkaline liver extracts inactivated stilbestrol but failed to inactivate estrone. Werthessen *et al.* (270, 271) called attention to the fact that the potency established for a substance when assayed by evaluation of the vaginal response is not necessarily the same when estimated by other methods. These workers investigated the effect of various natural and synthetic estrogens as inhibitors or stimulants of egg growth in the ovariec-

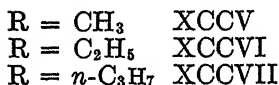
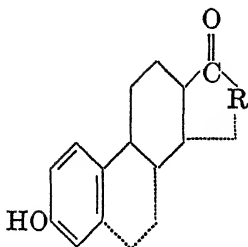
tomized rabbit, after progesterone administration; they also studied the effect on the uterine mucosa. A similar though inconclusive study had been undertaken by Fønss-Bech (241). The detailed discussion of their results is beyond the scope of this review. Nevertheless, some of the conclusions reached by Werthessen *et al.* are of great interest regarding the effect of alkylating the phenolic hydroxyl groups in synthetic estrogens. Under the conditions of the experiment stilbestrol was found to have an inhibitory effect on egg growth similar to that observed for estrone (CLXVII) but quite opposite to the effect of estradiol (XCV); stilbestrol monomethyl ether has qualitatively the same effect as stilbestrol. Quantitatively, however, the monomethyl ether was found to be four times as potent an inhibitor as the parent substance. As mentioned earlier, the ratio of potencies when assayed by the conventional evaluation of vaginal response is quite the reverse, i.e., 4:1 in favor of stilbestrol (85). The monomethyl ether further differs also qualitatively from stilbestrol in that it fails to inhibit the response of the uterine mucosa to progesterone stimulation. Werthessen and coworkers also demonstrated the potency of the monomethyl ether in stimulating the uterus on a short-time assay (6 hr.), thereby disproving the possible explanation of the differences between stilbestrol and its monomethyl ether by the prolonged effect of the latter. The authors concluded that the pharmacology of the two compounds is not the same. If this be true in general, one may have to revise the earlier picture of the alkyl derivatives acting only after physiological demethylation to the dihydroxy derivatives. That the latter process is not without significance was corroborated by the finding of Shorr and Mazur (as quoted by Werthessen and Gargill (270)) that the excretion product of the monomethyl ether is stilbestrol, presumably in the form of the glucuronide.

Contrary to the results obtained by Sondern *et al.* (198), Jaap and Thayer (244) found that stilbestrol dimethyl ether was much more active than stilbestrol, its diethyl ether, or its dipropionate, when tested orally in the domestic fowl. After extending this investigation to other estrogens and to other species, Jaap (243) concluded that so far no consistent relation may be established between the oral activity and the state of the various estrogens regarding methylation of the phenolic hydroxyl groups.

Zima *et al.* (272) investigated the mode and duration of activity of stilbestrol and its esters in humans. The former was excreted completely within 24 hr., while the retention time of the esters in the organism depended on their rate of hydrolysis. These workers concluded that there is no basic difference between the parent substance and its esters regarding the mode of resorption or action.

XII. APPENDIX

It is worth noting an unsuccessful attempt to prepare synthetic estrogens resembling the natural estrogens in a manner different from that of stilbestrol. Johnson and Offenbauer (246) synthesized 4-(*p*-hydroxyphenyl)hexahydroacetophenone (XCCV) and the two higher homologs (XCCVI and XCCVII).



In spite of the resemblance, as indicated by the dotted lines, all compounds showed no estrogenic activity (245).

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